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BACTERIOLOGIC STUDIES ON THE FECAL STREPTOCOCCI AND THE LACTIC ACID STREPTOCOCCI*

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MANY workers seem to be of the opinion that there is a great deal of overlapping in the classification of certain species of bacteria. A number of species are described and named which, from their general reactions, apparently do not differ greatly from other species classified in the same genus.

With this in mind, and the fact that numerous references in the literature have stated that *Streptococcus fecalis* and *Streptococcus lactis* are or seem to be identical, this work was begun. The purpose was to study a number of organisms of each species simultaneously, subject them to the same conditions of growth, and carry out various tests in order to note any differences in their reactions which would warrant classification as separate and distinct species.

Some of the earlier workers who noticed the similarity between *Streptococcus fecalis* and *Streptococcus lactis* were Kruse,[1] Sittler,[2] and Schmitz.[3] Later, in 1924, S. H. Ayers and W. T. Johnson, Jr.,[4] became interested in this matter when they noted a similarity of reactions between the fecal streptococci and their cultures of *Streptococcus lactis.* They formulated the conclusion that *Streptococcus fecalis* or enterococcus, "is similar to, if not identical with, *Streptococcus lactis.*" They further claimed that the question still remained unanswered but more clearly understood.

Schmitz,[5] Meyer and Schonfeld,[6] Callerio,[7] and Gundel,[8] each working on either fecal or lactic acid streptococci, state that it is their belief that these two organisms are identical. Karl J. Detmeter, in 1929,[10] stated that "there is no

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real difference between *Streptococcus lactis* and *Streptococcus fecalis* and that the name *Streptococcus lactis* may be used to define a local form of *Streptococcus fecalis,* more or less adapted to milk medium."

Grossman[11] states that the question of whether *Streptococcus lactis* and *Streptococcus fecalis* are identical needs further investigation.

There are numerous references in the literature pertaining to the use of the term *Streptococcus fecalis* or enterococcus. Thiercelin[12] found a gram-positive diplococcus in the intestine, in normal stools, and in purulent discharges, which he named enterococcus. Although the description which he gave the organism does not agree with later descriptions of an organism given the same name, the later description is usually the one considered when using the term enterococcus, i.e., large, oval-shaped cocci, appearing in pairs or short chains, capable of fermenting "sugars," usually heat resistant, and generally not pathogenic for laboratory animals.

Andrews and Horder[13] gave the name *Streptococcus fecalis* to an organism found in great numbers in the intestinal tract. Dible,[14] who has made an extensive study of the enterococci, claims that *Streptococcus fecalis* of Andrews and Horder is of the same group as the enterococcus, while *Streptococcus enteritis* (Libman) is a true chain-forming coccus of the type found in the oral cavity, and may be a surviving form of this organism. The terms *Streptococcus fecalis* and enterococcus are used hand-in-hand in this paper to denote the same organism.

The major cultural characteristics of members of the *Streptococcus fecalis* and *Streptococcus lactis* groups are given in Tables I and II, which were made up from the summaries of the various papers by the authors mentioned.

SOURCE OF CULTURES AND ISOLATION

The cultures for this study were isolated directly from feces and milk or milk products, such as buttermilk and cottage cheese.

Isolation.—Lactic Acid Streptococci: Numerous samples of unpasteurized milk, buttermilk, and cottage cheese were obtained. The buttermilk and cheese samples were plated out immediately upon receiving, in dilutions of 1:1,000 and 1:10,000 on bromthymol blue dextrose agar. Part of each sample was also streaked directly on bromthymol blue dextrose agar and incubated at 30° C. until colonies of streptococci were visible. The milk samples were allowed to stand at room temperature until the acidity reached approximately 0.2 per cent. They were then plated out on bromthymol blue dextrose agar in a dilution of 1:1,000 and 1:10,000. The plates were incubated at 30° C. until growth occurred. When the samples kept at room temperature reached an acidity of 0.4 per cent and finally 0.5 or 0.6 per cent, they were again plated out as described above. This procedure in principle is similar to that of Ayers, Johnson and Mudge,[19] who feel that by plating out at various acidities one is more apt to get the characteristic lactic acid streptococci.

When the colonies had grown out, they were examined for the presence of lactic acid streptococci. These appeared as small, deep yellow or orange, smooth, raised and glistening colonies. A few were "fished off" and later con-

TABLE I

MAJOR CULTURAL REACTIONS OF *S. fecalis* AS NOTED BY VARIOUS WORKERS

	MORPHOLOGY	HEMOLYSIS	HEAT RESISTANCE	BILE SOLUBILITY	ACTION ON MILK	INDOL	AESCULIN	GELATIN	GLUCOSE BROTH	GROWTH		SUCROSE	LACTOSE	INULIN	RAFFINOSE	MANNITOL	SALICIN	ARBUTIN	DEXTROSE	MALTOSE	GLYCEROL
										10° C.	45° C.										
Callerio[7] 1926	Pleomorphic cocci	–	+	–	Coag.	–										+	+	+			
Went[16] 1926	Gm. + oval diplo- str.	–		–	Acid, no coag.	–		+ after long time								"Does not ferment various sugars"					
Gordon[15] 1922		–													–	+					
Grossman[11] 1929	Oval or lancet	–	+	–			+														
Andrews and Horder[13] 1906	Short chained cocci	–										+	+	±	±	+	+				
Alston[17] 1928	Oval, pairs, and short chains	–	60° C. for 10 mins.									+	+	±		±	+		+		
Meyer[23] 1926		Beta at times	+	–			+									+					
Sherman and Stark[21] 1931		Gamma			Red. of litmus before coag.				pH 4.2	+	+	±	+	–	–	±	+		+	+	–
Bergey[28] 1930	Gm. + cocci oval				Acid, coag.			–				+	+	–		+	+		+	+	
Crowe[29] 1924	Short chained cocci		+									+	+			+	+				

TABLE II

MAJOR CULTURAL REACTIONS OF *S. lactis* AS NOTED BY VARIOUS WORKERS

	MORPHOLOGY	ACTION ON MILK	GROWTH AT 10° C.	GROWTH AT 45° C.	GROWTH AT 22° C.	REDUCTION OF JANUS GREEN OR AMMONIUM MOLYBDATE	LOW FINAL pH IN GLUCOSE BROTH	REDUCTION OF METHYLENE BLUE	SUCROSE	LACTOSE	MALTOSE	DEXTROSE	MANNITOL	SALICIN	RAFFINOSE	INULIN	LEVULOSE
Ayers, Johnson, and Mudge,[19] 1924	Oval cocci chains 2-6	Acid, red. of litmus coag.	+		+	+		+	+[1]	+	+	+	+[1]	+[1]			
Sherman and Albus,[18] 1918	Cocci, no chains on agar or broth	Red. of litmus before coag	+	+			+	+	+[1]			-					
Jones,[20] 1921																	
First group	Coccoid orgs., pairs, or chains	Reduce litmus coag. milk			+		+		1	+	+	+	1	+	1	1	
Second group									+	+	+	+	+	+	1	1	
Third group									+	+	+	+	1	+	1	+	+
Bergey,[28] 1930	Gm. +, cocci, pairs, or chains	Rapid coag. red. litmus							+[1]	+	+	+				+	+

firmed by Gram's stain, to be gram positive, diplococci, appearing in pairs and few very short chains. After several replatings the cultures were put in stock.

Fecal Streptococci: Samples of feces from both normal and sick human beings were obtained and a portion of the sample was emulsified in a tube of infusion broth and incubated at 30° C. for a few hours. Bromthymol blue dextrose agar plates were streaked with some of this material and incubated at 30° C. until growth appeared. The colonies of the fecal streptococci appear the same as the lactic acid streptococci on these plates.

Some of the material from the fecal specimens was placed in tubes of litmus milk and heated to 60° C. for fifteen minutes. These tubes were then incubated at 30° C. until acid production and coagulation occurred. From these tubes, bromthymol blue dextrose agar plates were streaked and incubated at 30° C. until colonies of fecal streptococci appeared. This method is similar to those of Dible[14] and Ayers and Johnson.[4] A few of the cultures were obtained by streaking some of the fecal material directly on bromthymol blue dextrose agar plates and incubating them until colonies of fecal streptococci appeared. The cultures were replated several times to insure purity and then placed in stock.

EXPERIMENTAL

The purpose of this work was not so much to use new tests, as to utilize those recommended by the various workers mentioned in the beginning of this paper, to try to duplicate their results, and finally, to see what conclusions could be drawn from the assembled results.

Fifty cultures of the lactic acid and fifty of the fecal streptococci were selected for the experimental work. The following studies were made.

CULTURAL CHARACTERISTICS

It was impossible to differentiate between the two types of streptococci when grown in either infusion broth, 0.1 per cent dextrose infusion broth or on blood agar or bromthymol blue dextrose agar. Morphologically they were pleomorphic, varying in size and shape. The cocci were usually oval or lemon shaped, with an occasional round form. Generally there were no long chains produced, except by a few strains when grown in 0.1 per cent dextrose infusion browth.

Culturally, both types grew well on all four media mentioned above. On blood agar they varied in the type of hemolysis produced, the majority of the strains giving the Alpha type. All strains grew well on bromthymol blue dextrose agar, forming characteristic colonies.

Bromthymol blue dextrose agar was made according to the formula of Small and Kreidler,[22] except that dextrose was used instead of lactose, and the pH was adjusted to 7.5. Dextrose seems to give a sharper colony and a deeper color. This medium is valuable in the primary isolation of members of either type of streptococci, since they produce characteristic colonies. Other dextrose fermenters produce larger colonies and the coloration is not quite as deep yellow.

The cultures, when grown in litmus milk, all produced acid. With the exception of six strains, they all reduced the litmus before coagulation. The

TABLE III*

MORPHOLOGIC AND CULTURAL CHARACTERISTICS OF FECAL STREPTOCOCCI AND LACTIC ACID STREPTOCOCCI

	NUMBER OF STRAINS	METHODS OF ISOLATION	MORPHOLOGY	HEMOLYSIS	GELATIN: GROWTH 20° C.	GELATIN: LIQUEFACTION	HEAT RESISTANCE, 60° C. FOR 20 MINUTES	LITMUS MILK	REDUCTION OF AMMONIUM MOLYBDATE	REDUCTION OF JANUS GREEN	REDUCTION OF METHYLENE BLUE IN MILK	BILE SOLUBILITY	H_2S PRODUCTION	ACID PRODUCTION AT 10° C.	ACID PRODUCTION AT 45° C.
Fecal Str.	16	Plates streaked from broth emulsions	Oval cocci, pairs and short chains	Gamma (10) Alpha (6)	+	(2) + (14) −	(14) + (2) −	(15) Typical (1) Atypical	(9) B (7) T	(2) ++ (7) + (7) −	(15) + (1) −	−	+	(4) 1.71 to 6.58 (12) −	4.18 to 4.28
	32	Heating and incubation in litmus milk	Oval cocci, no chains	Gamme (10) Alpha (22)	+	(4) + (28) −	(27) + (5) −	(29) Typical (3) Atypical	(17) B (15) T	(3) ++ (14) + (15) −	(26) + (4) R (2) −	−	+	(4) 4.62 to 6.69 (28) −	4.15 to 4.40
	2	Direct plating	Oval cocci, no chains	Alpha	+	−	+	Typical	B	+	+	−	+	−	4.30 4.32
Lactic Acid Str.	35	1:100 and 1:10,000 dilution plated	Oval cocci, no chains	Alpha	+	(6) + (29) −	(31) + (4) −	Typical	T	(31) ++ (4) +	(29) + (4) R (2) −	−	+	(31) 4.42 to 6.09 (4) −	4.10 to 4.66
	10	Plating direct on BTB agar	Oval cocci, pairs, few short chains	Alpha	+	(3) + (7) −	+	Typical	T	(6) ++ (4) +	(6) + (3) R (1) −	−	+	(4) 4.40 to 5.84 (6) −	4.25 to 5.64
	5	Streaking direct on BTB agar	Oval cocci, no chains	Alpha	+	(1) + (4) −	+	(3) Typical (2) Atypical	T	(3) ++ (2) +	(4) + (1) R	−	+	4.60 to 6.35	4.47 to 4.69

*Reduction of Ammonium Molybdate.
T, Blue color throughout the tube.
B, Blue color only in butt of tube.
Reduction of Janus Green.
++, Red color.
+, Purple color.
−, Blue color.

Reduction of Methylene Blue in Milk
+, Reduction of M. B. and coagulation.
R, Reduction of M. B. only.
−, M. B. not reduced.

typical reaction, acid, reduction of litmus but for a narrow pink band at the surface of the medium, and coagulation, was noted by Ayers and Johnson,[4] Sherman and Stark,[21] and others to be characteristic of either type of streptococci. Here it was noted that the lactic acid strains brought about this typical reaction much more rapidly than did the fecal strains.

TABLE IV

SUGAR FERMENTATIONS

SUGAR	FECAL STREPTOCOCCI			LACTIC ACID STREPTOCOCCI		
	NO. OF STRAINS PRODUCING ACID	pH RANGE OF ACID FORMERS	NO. OF STRAINS PRODUCING NO ACID	NO. OF STRAINS PRODUCING ACID	pH RANGE OF ACID FORMERS	NO. OF STRAINS PRODUCING NO ACID
Arabinose	45	4.30-6.96	5	3	5.11-6.94	47
Dextrin	50	5.26-6.57	0	42	5.28-6.98	8
Dulcitol	0		50	25	6.69-6.86	25
Galactose	50	4.46-5.26	0	50	4.54-6.07	0
Glycerol	50	4.83-6.53	0	46	4.54-6.94	4
Inulin	37	6.55-6.96	13	28	6.55-6.96	22
Lactose	50	4.25-5.00	0	50	4.58-6.18	0
Levulose	50	4.08-5.07	0	50	4.30-5.12	0
Maltose	50	4.12-5.02	0	50	4.26-5.01	0
Mannitol	42	4.25-5.64	8	27	4.66-5.39	23
Raffinose	44	4.37-6.97	6	9	5.17-6.98	41
Salicin	50	4.15-5.18	0	34	4.35-5.00	16
Sorbitol	50	4.33-6.94	0	50	4.37-5.47	0
Sucrose	41	4.25-6.66	9	46	4.49-5.11	4

TABLE V

FERMENTATION OF DEXTROSE

DROP IN pH NOTED EVERY OTHER DAY FOR A PERIOD OF THREE WEEKS

FECAL STREPTOCOCCI										LACTIC ACID STREPTOCOCCI									
3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st	3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st
4.24-5.26	4.00-5.03	4.00-5.03	4.00-5.03	4.00-5.03	3.96-5.03	3.96-5.03	4.00-5.07	4.05-5.07	4.07-5.12	3.98-6.18	4.15-6.18	4.15-6.18	4.10-6.15	4.07-6.10	4.17-6.10	4.17-6.10	4.20-6.05	4.12-6.05	4.26-6.01

The medium used for the fermentation studies was similar to that recommended by Ayers and Johnson.[4] The sugars were sterilized separately in 5 per cent aqueous solutions and then added to the tubes to make a final sugar concentration of 0.5 per cent. The cultures were incubated for seven days and the change in pH noted with the use of the quinhydrone potentiometer. Dextrose fermentations were tested every other day for a period of three weeks, to determine when the lowest pH was reached.

Ayers and Johnson[4] claim that *Streptococcus lactis* seems to reach a lower final pH than the fecal streptococci. It was found that the fecal strains produced a lower pH than the lactic acid groups and that they were more constant in their acid production, showing usually a smaller range in pH. A number of the lactic acid strains caused a rise in pH during their growth in some sugars, reaching in a few cases a pH of 8.02.

On the basis of group majority it was found that the fecal groups fermented arabinose, mannitol, raffinose and salicin. A very small majority of the lactic acid strains fermented mannitol and salicin and only a few strains fermented arabinose and raffinose. Dulcitol was not fermented by any of the fecal strains but by half (25) of the lactic acid strains. The remainder of the sugars as indicated in Table IV were fermented by nearly the same proportion of strains in either type.

In the fermentation of dextrose it was found that the lactic acid organisms generally reached a lower pH much sooner than the fecal strains. They also reverted back to alkaline sooner, and in three weeks showed a higher pH than the fecal strains.

Tests made to determine the amount of variation between parallel fermentations by the same strains, in regard to the drop in pH, showed that a difference of 0.03 to 0.09 of a pH unit could be expected. This difference was even greater in cases where the sugar was not readily fermented by the organism.

It is generally considered that *Streptococcus fecalis* will not grow at 10° C., although Ayers and Johnson[4] and Sherman and Stark[21] found that their strains did grow at that temperature. Here it is noted that when the strains were grown in dextrose infusion broth there were not very many which grew and produced acid at 10° C. This may have been due to two factors: namely, first, that the medium used was not a satisfactory one for the test; second, that under the conditions available it was impossible to maintain a constant temperature of 10° C., it usually varied from 6 to 8° C.

Sherman and Stark,[21] together with other workers, state that it is generally felt that *Streptococcus lactis* will not grow at 45° C., and that this test might be used as a means of differentiating the fecal and the lactic acid streptococci. It was noted here, however, that both types grew well and produced acid at 45° C., and further, that no distinction between them could be made on this basis.

In testing for the reducing ability of the strains, the three methods used by Ayers, Johnson and Mudge,[19] namely, reduction of Janus green, reduction of ammonium molybdate and the reduction of methylene blue in milk, were attempted.

Both types possess fairly strong reducing abilities, the majority of strains reducing the various dyes. In the reduction of Janus green the lactic acid strains seemed to have the greatest effect. However, in the reduction of ammonium molybdate, the condition was just the reverse, in that the fecal groups appeared to have the stronger reducing power. The reduction of methylene blue was fairly rapid in both types. The majority of the strains completely reduced the dye and coagulated the milk. A few strains of either type failed to reduce methylene blue or reduced it for a short period, after which the color came back throughout the tube.

In testing for heat resistance and bile solubility, the methods used by Alston[17] were followed. Bromthymol blue dextrose agar plates were used to grow the organisms after the exposure to 60° C. for the required time, since it was found that this medium served as a better guide to determining the numbers

surviving, than did blood agar plates. This was due to the fact that the medium turns yellow when acid is produced in large amounts.

The majority of strains were resistant to 60° C. for twenty minutes. However, in a few cases the organisms failed to withstand this temperature for a longer period than ten minutes. Ayers and Johnson[4] found that their strains withstood this temperature for thirty minutes.

All strains were insoluble in bile when tested in the three concentrations of 10 per cent sodium taurocholate, namely: 0.2 c.c., 0.1 c.c., and 0.05 c.c. to 1 c.c. of a twenty-four-hour broth culture. The pneumococcus controls were all soluble in these three amounts.

Tests for growth and liquefaction of gelatin showed that a few strains of either type were able to liquefy the gelatin. These strains were positive in three days and none of the other strains showed any liquefaction up to three weeks' incubation at 20° C. All strains grew well in this medium. The tubes were placed in the refrigerator before a final reading.

The production of hydrogen sulphide was noted by following the formula given by Ayers and Johnson.[24] All strains produced hydrogen sulphide when some sulphur compound was added to the basic medium. It was impossible to differentiate between the two types on this medium.

Serologic Reactions.—Agglutinations: Five strains of each type were selected for agglutination on the basis of their differences in the various tests as described above.

Preparation of Monovalent Rabbit Serums: An infusion broth was prepared containing 1 per cent peptone, to which was added 1.03 gm. of disodium acid phosphate and 0.12 gm. of potassium dihydrogen phosphate per liter. The reaction was adjusted to pH 7.6 and the medium dispensed in 40 c.c. amounts in small flasks, and sterilized at 15 pounds for fifteen minutes.

Forty-eight-hour cultures grown in this broth were centrifuged and resuspended in saline. This process was repeated several times. For use the cells from 40 c.c. of culture were suspended in about 25 to 30 c.c. of physiologic saline and killed by heating in a water-bath at 60° C. for one hour.

The rabbits were injected first subcutaneously on consecutive days, in 0.25, 0.5, and 1.00 c.c. amounts. A rest period followed, after which they received 0.25, 0.5, and 0.5 c.c. of each suspension intravenously. After a rest period of three or four days they received three more intravenous injections in just slightly larger amounts than previously used. This procedure was kept up for six weeks, each time injecting slightly larger amounts of the antigens, reaching a maximum of 2.0 c.c.

The serum of each rabbit was tested at intervals during the period of injections for agglutination titer. One week after the last series of injections the rabbits were bled from the heart, the serum collected and preserved in 0.3 per cent trikresol.

Agglutination: Tests were set up using homologous antigens, both with suspensions of live bacteria and those killed for the injections. It was found that the results were the same in either case; therefore, live suspensions were used throughout the remainder of the work.

The antiserums were set up in dilutions ranging from 1:10 to 1:10,240, using 0.85 per cent NaCl for dilutions. To each tube, including a control, was added 0.5 c.c. of the homologous antigens. Controls of the various antigens and normal rabbit serums were set up along with the above tests. The tubes were placed in a water-bath at 52° C., read after three hours, and confirmed after eighteen hours in the water-bath. Cross-agglutination tests were set up to note whether there was any evidence of grouping. The titer of each serum, together with the results of the cross-agglutination tests, are shown in Table VI.

Both types are characterized by their ability to cross-agglutinate. This was noted by other workers, but especially by Hucker,[26] who found that the "serum of *Streptococcus faecium* (Orla-Jensen) agglutinated strains of *Streptococcus lactis.*" It was noted that a division between these two types on the basis of agglutination tests would be difficult, hence agglutinin absorption tests were tried.

TABLE VI

RESULTS OF AGGLUTINATION AND CROSS-AGGLUTINATION TESTS WITH ANTISERUMS OF FIVE OF THE FECAL AND FIVE OF THE LACTIC ACID STREPTOCOCCI

	F 3	F 8	F 19	F 51	F 53	L 3	L 15	L 23	L 41	L 43	NORMAL RABBIT SERUM
F 3	**5120**	2560	2560	0	0	2560	160	0	1280	0	0
F 8	2560	**5120**	2560	0	0	0	0	0	1280	0	0
F 19	2560	2560	**5120**	0	0	0	0	0	1280	0	0
F 51	0	0	0	**1280**	0	0	0	0	0	0	0
F 53	0	0	0	0	**2560**	0	0	0	0	0	0
L 3	0	0	0	0	0	**1280**	160	0	0	0	0
L 15	0	0	0	0	0	0	**2560**	0	0	0	0
L 23	0	0	0	0	0	0	0	**2560**	0	0	0
L 41*	----	----	----	----	----	----	----	----	----	----	----
L 43	0	0	0	0	0	0	160	0	0	**2560**	0

*L 41, one of the lactic acid strains auto-agglutinated continuously, hence no results could be obtained on its titer or on its cross-agglutinative ability.

Agglutinin Absorption: F 3, F 8, F 19, L 3, and L 15 serums were diluted 1:50 with a heavy suspension of each strain which showed cross-agglutination with them. These diluted serums were kept at 37° C. for four hours, with occasional shaking. They were then placed in the refrigerator overnight, after which they were centrifuged and the clear fluid used to set up agglutination tests with their homologous antigens. The change in titer was noted and recorded.

There were some evidences of absorption of agglutinins, although the test failed clearly to differentiate the types. F 3, F 8, and F 19 serums appeared to be the same in the cross-agglutination tests, but in the absorption reaction they show up as distinct variants with a common agglutinin. F 3, L 3, and L 15 possess a common agglutinin together with small amounts of specific agglutinins. L 15 seems to possess a predominating agglutinin which is also present in L 3 and L 43 in small amounts.

On the whole, the agglutination and the agglutinin absorption reactions were not of much value in attempting to show a clear distinction between the two types of streptococci. This is mainly due to the contradictory results obtained in cross-agglutination, and, perhaps, as stated by Hucker,[26] to a decided strain

specificity of some of the organisms. It is felt, however, that these strains are all serologically related, and that there exist several distinct variants.

Numerous attempts were made to secure an antigen of L 41 which would not auto-agglutinate, all of which were unsuccessful. The procedure recommended by Crowe,[27] was used in several attempts with some of the other strains which at times would auto-agglutinate. This method, however, failed in the case of L 14, which, if it showed no auto-agglutination before the test was set up, always spontaneously agglutinated after incubation, as evidenced by the control tubes.

DISCUSSION

As noted in Tables I and II, there seem to exist some differences of opinion as to which reactions constitute the basic characteristics of these two types of streptococci. Variations in technic and tests used would tend to give some varied results. This perhaps accounts for the differences in opinion in regard to the relation of these two types of organisms. The types of media and the initial pH, it is felt, are two very important factors in attempting to correlate results when working with streptococci. A standardization of methods and technic would go far in eliminating many of the present discrepancies in respect to this field. Numerous workers have attempted this matter, and it remains now to select those which will bring out the important physiologic characteristics of the organisms.

These two types of organisms resemble each other very closely. There are slight differences but they may be attributed to the fact that the changes in environmental conditions would tend to bring about a change of some of the physiologic reactions, producing some of the variable characteristics which they possess. Their pleomorphic traits also suggest this.

Sherman and Albus, Jones, and numerous other workers have shown that *Streptococcus lactis* is not an inhabitant of the udder and that it is not found in freshly drawn milk. It must enter the milk from some outside source, in very small numbers, for it cannot readily be isolated from milk less than thirty-six to forty-eight hours old. Since it is well adapted to a milk medium, it soon becomes the predominating organism and remains as such until the acidity becomes too great, when it again diminishes in numbers.

SUMMARY AND CONCLUSIONS

Fifty strains each of members of the fecal streptococci and the lactic acid streptococci were studied simultaneously for any morphologic, cultural, or serologic characteristics which would warrant their being classified as two distinct species.

It was the purpose of this study to assemble the most important methods and tests generally in use or those used particularly by various authors and note whether any differences in reactions occurred between these two types of streptococci. Variations in technic or new methods were employed only where it was impossible to carry out the original procedure.

Detailed studies were made on the growth and action in various media. Finally, immune serums were prepared by rabbit inoculation. Agglutination, cross-agglutination, and agglutinin absorption tests were set up.

It is felt that there exists no real difference between these two types of streptococci. Slight variations which occur are not of real importance since they may occur not only between the two types but also among the groups themselves. These slight variations perhaps are due to the fact that the organisms undergo extreme changes in environmental conditions.

Since the lactic acid streptococci appear to be able to withstand conditions as they occur in the animal body, it is reasonable to assume that they should be found in the intestinal tract. As indicated here and also by other workers, there seems to exist no real difference between the fecal streptococci and those normally found in milk. Therefore, it is felt that the *Streptococcus lactis* ingested with milk and some milk products is of the same type as that found in the feces. From here they may enter the soil where only the most vigorous can survive and in turn enter milk through sources of contamination.

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THE QUININE TEST FOR HYPERTHYROIDISM*

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WHILE the recognition of typical exophthalmic goiter or Graves' disease is devoid of difficulties, the many atypical cases in our midst require laboratory tests for confirmation of diagnosis. We are still puzzled by the large percentage of otherwise typical cases presenting no exophthalmos or no thyroid swelling. Indeed, in approximately 11 per cent of our series of cases both exophthalmos and goiter were absent at the time of the primary examination.

TESTS FOR HYPERTHYROIDISM

Aside from the basal metabolism test, which, properly performed under ideal physical, mechanical, and psychologic conditions, is still quite dependable in the majority of cases, there are many other tests that have been discussed in the literature from time to time. Among these are the adrenalin test, the Kottman test, the blood-iodine and blood cholesterol tests, the erythrocyte sedimentation test, the velocity of the blood flow test, the impedance angle test, and the quinine tolerance test to which I called attention in 1920 and in later communications.[1, 2, 3] Many of these tests possess genuine clinical value. However, one need not go far afield to seek for reliable means of diagnosis of atypical exophthalmic goiter. In our experience the most dependable means are first, the experience and five senses of the doctor himself, second and third, the basal metabolic and the quinine tolerance tests.

The Quinine Test.—Continuing our studies prior to 1920, we found in a series of over 4,000 cases of hyperthyroidism (with and without Graves' disease) that over 95 per cent tolerated from 30 to 90 gr. of quinine sulphate daily for weeks without evidences of cinchonism. The relative immunity to cinchonism exhibited by these patients lent itself to the adoption of the quinine test for hyperthyroidism, the technic of which is as follows:

The patient is given a dozen capsules, each containing 10 gr. (0.65 gm.) of quinine hydrobromide (or the sulphate), with instructions to take a capsule 3 times a day. If after

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four days there are no evidences of cinchonism, the individual's reaction may be considered positive for hyperthyroidism. When 20 or 30 gr. (1.3 or 1.9 gm.) have been taken by persons in whom thyroid function is normal or deficient, symptoms of cinchonism develop. A sense of fullness in the head, impaired hearing with tinnitus, occasional dizziness and headache, and less frequently, gastric and bladder discomfort are experienced. If, however, the person is suffering with hyperthyroidism, the daily administration of 30 gr. of quinine may be continued with impunity for months, and often with distinct improvement in the clinical picture.

In our series 4.25 per cent were quinine negative. Forty-five presented varying degrees of quinine sensitivity. In the remainder the tolerance for quinine varied in dosage from 30 to 90 gr. a day for at least three weeks in each case.

Male patients predominated among those able to take the largest doses of quinine. Also, following the ingestion of the drug, there occurred in at least 60 per cent of this series such evidences of general improvement as increased weight, lessened excitability and tremor, improved sleep, and a reduction in the heart and basal metabolic rate. The advantages of quinine in treatment were outlined in another contribution.[4]

The merits of the quinine test are its simplicity and relative dependability. The results are not influenced by emotionalism nor are such factors as transient nervousness and errors in technic involved. Moreover, the test requires no "basal conditions" for its use, the patient requiring no preliminary period of rest or starvation.

The quinine test does not discriminate between toxic adenoma and exophthalmic goiter. This differentiation requires not laboratory but clinical deductions. In a general way, however, we feel that the average patient with exophthalmic goiter is somewhat more tolerant to quinine than is the average sufferer from toxic adenoma.

TABLE I

TOLERANCE FOR QUININE IN 4,000 CASES OF HYPERTHYROIDISM WITH AND WITHOUT GRAVES' DISEASE

Quinine Positive:		
Patients tolerating 30 gr. daily	1,334 or	33.4 per cent
Patients tolerating 40 gr. daily	836 or	20.9 per cent
Patients tolerating 60 gr. daily	725 or	18.1 per cent
Patients tolerating 80 gr. daily	527 or	13.2 per cent
Patients tolerating 90 gr. daily	408 or	10.2 per cent
Quinine Negative:		
Patients sensitive to quinine	45 or	1.1 per cent
Patients tolerating 20 to 30 gr. daily	125 or	3.1 per cent
Total	4,000 or	100.0 per cent

QUININE NEGATIVE CASES

It appears that in at least 95 per cent the element of hyperthyroidism must be present in order that the quinine test be positive and a relative immunity to cinchonism be expected. In other words, patients able to tolerate 30 or more grains of quinine hydrobromide or quinine sulphate a day for a few days or longer present the most important evidence of hyperthyroidism, namely, a

heightened basal metabolism rate. This was emphasized when it was observed that, barring the occasional instance of quinine sensitivity in this series, most of the patients with mild evidence of cinchonism (i.e., slight fullness in the head and tinnitus) were sufferers from the uncommon type of Graves' disease with a normal basal metabolism rate. Of late years clinicians who see many cases of Graves' disease realize that a heightened metabolic rate is occasionally conspicuous by its absence in an otherwise typical syndrome. Despite heart hurry, trembling, loss in weight, exophthalmos, emotionalism, and nervousness, in some instances of active Graves' disease the element of hyperthyroidism per se may not be demonstrable. In our experience approximately 1.5 per cent of subjects of Graves' disease present no rise in metabolism and are likely to be quinine-negative. Among these are included cases of thyroid quiescence due to remission of the syndrome following thyroidectomy, roentgen ray treatment, iodine administration, or as a result of spontaneous involution. While exophthalmos, heart excitability, tremor and fatigability may still prevail to an extent, the thyroid gland is not in a state of hyperactivity.

QUININE TOLERANCE BY THYROID NORMAL PERSONS

The tolerance to quinine by sufferers from malaria and from such febrile conditions as pneumonia, acute tonsillar diseases, and other infectious processes must not be construed as a state of natural immunity from the effects of the drug, but as a temporarily altered condition of the bodily reactions, to return to normal on the recovery of the patient. These instances of tolerance are easily determined and need not detract from the reliability of the quinine test in hyperthyroidism. In quinine-negative persons it is found that after taking 2 or 3 capsules of the quinine salt each containing 10 gr. at approximately five-hour intervals, the typical head and ear symptoms of cinchonism develop, indicating intolerance to this dosage. In order to ascertain the amount of quinine tolerated by normal controls, we succeeded in enlisting the services of eight apparently normal adults of average size and weight, varying in age between twenty and forty-one years. Four were men and four were women. The period of observation was two weeks in each subject. Head and ear symptoms were indices of intolerance, and quinine sulphate was given in evenly divided doses five or six hours apart, t.i.d. Two subjects were able to tolerate 12 gr. daily; three could take but 9 gr. a day; two could not exceed 6 gr.; and one developed cinchonism when the dosage exceeded 3 gr. daily. According to this group the average normal person can tolerate 8.25 gr. of quinine sulphate a day for two weeks without experiencing symptoms of cinchonism.

VARIATIONS IN QUININE TOLERANCE IN THE SAME PATIENT

In the average case of hyperthyroidism quinine tolerance appears to vary directly with the severity of the symptoms. The degree of tolerance runs fairly parallel with the heightening of the basal metabolism rate. Early or mild cases usually tolerate 30 gr. or more of quinine hydrobromide or sulphate daily in the presence of a basal metabolism rate varying from plus 30 to plus 40 per cent.

In the average sufferer with a metabolic rate of plus 50 or over, and during crisis, quinine tolerance may be increased to even 90 gr. daily and, in a few instances in our series, even higher.

When, while under treatment, a patient formerly tolerant to large doses of quinine now begins to complain of head and ear symptoms, it is invariably found that the basal metabolism rate, too, is approaching or has become normal. Formerly capable of tolerating large doses of quinine daily, such a patient now gradually becomes quinine-negative. The degree of quinine tolerance manifested by the individual is a fairly satisfactory index of the degree of thyroid hyperfunction. While precise figures have not yet been worked out, further investigation may determine the quinine tolerance of a given subject of hyperthyroidism to be quite as reliable an index of the presence or severity of the disease and of the progress toward recovery as is the basal metabolic rate.

Relative immunity to cinchonism obtains only during the time the individual is suffering from the disease, the amount of quinine satisfactorily tolerated by the patient running fairly parallel with the height of the basal metabolic rate. When, having been capable of taking 30 or more grains of quinine hydrobromide or sulphate with impunity for weeks or months, the patient complains of buzzing in the ears and roaring in the head, it will be found that the heart and metabolic rates and sense of well-being have become quite normal. At this point those clinicians employing the drug may continue giving quinine in doses tolerated without cinchonism, which in the average individual may vary from 3 to 10 gr. a day. This dosage is suggested for two or three months, when it may be discontinued.

SENSITIVITY TO QUININE

Sensitivity to cinchonism and its derivatives may occur where least expected, so that when administering quinine either as a test or as a therapeutic measure, the medical attendant should see the patient daily for the first few days. In our series there were patients in whom intolerance was associated with more or less troublesome symptoms, particularly referable to the skin.

Cases of sensitivity to quinine can be apprehended with the Boerner test prior to quinine ingestion. A positive Boerner's reaction probably means that the patient will have discomfort from the administration of the drug; a negative reaction, on the other hand, does not preclude the occurrence of urticaria. Patients presenting an idiosynerasy to quinine may be found tolerant to quinidine. Boerner, himself sensitive to quinine, describes a positive test as follows:[5]

> The method of conducting the test is similar to the cutaneous tuberculin test (von Pirquet) with quinine sulphate as antigen. The forearm is cleansed with alcohol and dried. Two abrasions are made with a needle about 3 inches apart. The scarifications are just deep enough to remove the superficial layers of the skin. To one of these abrasions, powdered quinine sulphate is applied. The other is left untouched to show the amount of irritation produced by the traumatism. In about five minutes the abrasion to which the quinine has been applied begins to itch slightly. This is followed by slight edema along both sides of the pin mark which in ten minutes becomes very pronounced. At the end of fifteen minutes the reaction is at its height, and at this time is surrounded by a zone of erythema presenting a mottled appearance and extending over an area about 1 inch in diameter. Instead of powdered quinine sulphate, a solution of quinine bisulphate in 1:10 dilution with normal salt solution may be used.

CONCLUSIONS

1. From our observations on a series of over 4,000 cases it appears that the quinine test for thyrotoxemia is a dependable guide in diagnosis, the frequency of error not exceeding 5 per cent. As with basal metabolic studies, the test does not discriminate between toxic adenoma (true hyperthyroidism) and exophthalmic goiter (Graves' disease).

2. The tolerance for quinine by subjects of hyperthyroidism appears to vary in direct proportion with the height of the basal metabolism rate and is fairly parallel with it, serving as a guide in progress under treatment. Depending upon the severity of active hyperthyroidism, patients are capable of taking 30 or more grains of quinine sulphate or hydrobromide daily for weeks without evidence of cinchonism.

3. In the occasional instance of a quinine-negative subject who was an otherwise typical case of exophthalmic goiter, it was discovered that we were dealing with an uncommon case of Graves' syndrome, apparently without the element of hyperthyroidism. In these patients, despite nervousness, sweating, wasting, exophthalmos, heart hurry, trembling, etc., the basal metabolism rate remained within normal limits.

4. It appears that the quinine test is as dependable as the basal metabolism rate and as accurate a guide in treatment; it has the advantage of requiring no costly apparatus in its performance nor does it require "basal conditions" of starvation and rest.

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CONGENITAL THINNING OF THE WALL OF THE RIGHT ANTERIOR AORTIC SINUS OF VALSALVA*

Anterior Interventricular Septal Defect (probably bulbar septal), Slight Dextroposition of the Aorta and Bacterial Endocarditis

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A CASE of congenital thinning of the wall of the right anterior aortic sinus of Valsalva, anterior interventricular septal defect and dextroposition of the aorta, terminating with bacterial endocarditis, is the subject of this report. A close correlation was established between the embryologic development of the heart and the morphology of the defects on the one hand and between the autopsy findings and the clinical manifestations elicited by ordinary methods on the other. It is of additional interest and significance that the right orifice of the interventricular septal defect occupied a position behind the septal cusp of the tricuspid valve. This location would ordinarily have invalidated the classification of this defect as being bulboseptal in type had it not been for the dextroposition of the aorta, the typical situation of the left ventricular ostium of the defect and the associated congenital thinning of the wall of the right aortic sinus of Valsalva.

CASE REPORT

S. M., white male, aged sixteen years, complaining of headache, weakness, hematuria, and soreness of the left arm and leg of a week's duration, died the day following admission to the hospital (service of Dr. Thomas McCrae). There had been no history of sore throat, growing pains, chorea, or of acute rheumatic fever, and there had been no cyanosis, dyspnea, or edema. A chance physical examination at the age of two years revealed that the heart was not normal and the parents of the child were advised to restrict his exercise. Following admission, the third left toe became painful, red and discolored, a right facial palsy and flaccid paralysis of the left arm and right side of the tongue developed and petechiae appeared in the conjunctivae, and on the tip of the right index finger. On physical examination there was limited expansion of the left upper thorax and the left diaphragm did not appear to move as freely as the right. The cardiac impulse was widespread, wavy, diffuse, and rapid with the apex beat in the sixth left interspace, 13.5 cm. from the midsternal line. The right border of cardiac dullness extended 4 cm. from the midsternal line and a systolic thrill was palpable over the lower part of the thorax. On auscultation a loud systolic murmur was heard over the entire precordium transmitted into the neck and left axilla with its maximum intensity at the third interspace, to the left of the sternum. The temperature was 100° F., the pulse rate 110 per minute, the respirations 28 per minute, and the blood pressure 130 mm. mercury systolic and 90 mm. diastolic. The blood contained 80 per cent Hg., 4,000,000 erythrocytes, and 16,700 leucocytes; *Staphylococcus aureus* grew in blood culture. The urine contained blood and albumin.

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POSTMORTEM EXAMINATION (2 HOURS AFTER DEATH)

The combined gross and microscopic diagnoses were: (1) Congenital heart disease, anterior interventricular septal (probably bulbar septal), slight dextroposition of the aorta and bacterial endocarditis and congenital thinning of the wall of the right anterior aortic sinus of Valsalva; (2) subaortic stenosis; (3) endocardial systolic pocket of the left ventricle; (4) fenestration of posterior aortic cusp; (5) marked endocardial sclerosis, especially of the aortic cusps; (6) acute bacterial endocarditis of the aortic cusps and a portion of the endocardium of the right ventricle opposite the orifice of the interventricular septal defect; (7) septic infarction of the brain, kidneys, spleen, lungs, and third toe of the left foot; (8) widely distributed cutaneous, mucosal, and serosal petechial hemorrhages.

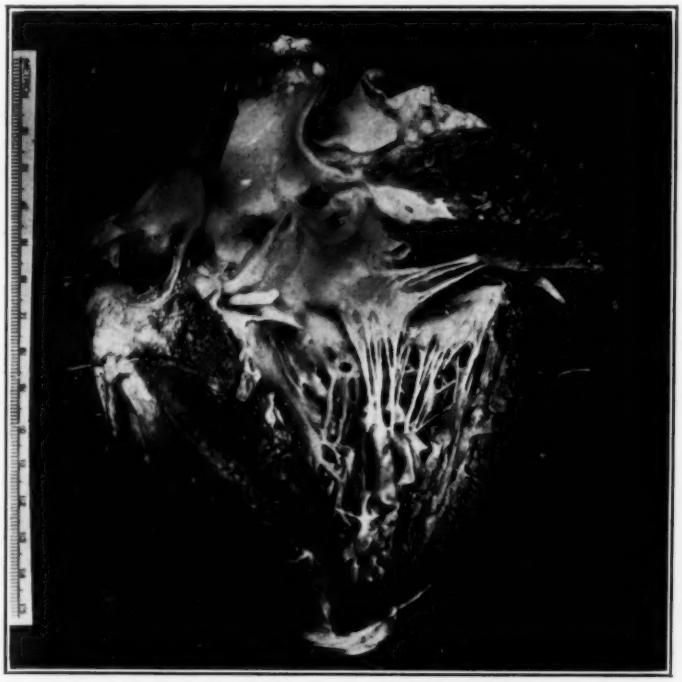

Fig. 1.—Heart showing interior of left ventricle, mitral leaflets, aorta and interventricular septal defect (probe). The left aortic cusp is shaped like a cup, lies above the anterior leaflet of the mitral valve, and presents a darkened area of thrombus formation. The posterior aortic cusp is markedly thickened and fenestrated in its left portion. The interventricular septal defect lies below the commissure of the right and posterior aortic cusps. A systolic endocardial pocket is situated upon the muscular prominence of the interventricular septum.

Postmortem bacteriologic studies: *Staphylococcus aureus* was recovered in pure culture from blood and from the substance of a splenic infarct.

DESCRIPTION OF HEART

The heart was large, showing hypertrophy of the walls of both ventricles, and sclerosis of the endocardium of the left auricle, mitral leaflets, aortic cusps, and subaortic area (Fig. 1). The superior portion of the interventricular muscular septum was unusually well developed and caused a slight degree of subaortic stenosis. The thick gray, opaque endocardium of this region formed a systolic endocardial pocket on the most prominent portion of this muscular ridge, the opening of the pocket being directed downward toward the apex of the ventricle. The aorta was rotated slightly backward and to the right and was continuous with the somewhat contracted chamber of the left ventricle which was traversed near its apex by a fibrous band resembling a chorda tendineae. The aortic cusps were thickened, nodular, adherent to each other and irregular in size and shape with inverted margins and

broad retracted stubby commissures. The left aortic cusp was situated upon the anterior leaflet of the mitral valve and presented a cuplike appearance with a wide base and narrow constricted orifice; there was a small oval, roughened plaque of fresh thrombotic material on the ventricular aspect of this cusp. On the lower part of the commissure of the right and posterior aortic cusps the endocardium was covered by a small amount of thrombotic material. Directly below this area and behind the superior ledge of the subaortic muscular shelf was an oval, funnel-shaped interventricular septal defect. This was lined with thickened endocardium and bordered anteriorly and inferiorly by muscular tissue while posteriorly it approached to within 1 cm. of the pars membranacea.

In the dilated right ventricle (Fig. 2) the septal defect presented a larger vertical, slitlike orifice beneath the septal insertion of the anterior tricuspid ledge and above and

Fig. 2.—Heart showing interior of right auricle and ventricle. The orifice of the interventricular septal defect opens behind the septal cusp of the tricuspid valve (probe). An area of mural endocarditis can be seen on the anterior tricuspid ledge directly above the probe.

posterior to the extremely small papillary muscle of Lancisi. A globular thrombus with a fan-shaped base radiated outward from the right orifice of the defect along the endocardium of the posterior midportion of the anterior tricuspid ledge. Above and anterior to the septal defect was a thin, diamond-shaped, membranous septum which separated the pulmonary conus from the aorta. The two superior margins of this septum were formed by the converging borders of the right and left pulmonic cusps while the inferior margins were bordered by two muscular bands of the conus. These latter extended downward to the papillary muscle of Lancisi and to within 0.6 cm. of the anterior margin of the interventricular septal defect, where they met to form the inferior angle of this area of congenital thinning. The aortic surface of this septum lay opposite the right anterior sinus of Valsalva.

The descending branch of the left coronary artery was narrowed at about the junction of the upper third and lower two-thirds by an inflammatory lesion lying deep in the epicardial

fat; the caliber of its lumen was further diminished at this point by a small amount of thrombotic material. Distal to this lesion the epicardium was dotted over a fan-shaped area with small petechial hemorrhages. The right coronary orifice was situated at the extreme right superior portion of the sinus of Valsalva, well above and to the right of the area of congenital thinning.

Weights and Measurements: The heart weighed 500 gm. and measured 15 by 10 by 7 cm. The thickness of its walls was as follows: left ventricle, 3 cm.; right ventricle, 0.8 cm.; each auricle, 0.2 cm. The circumference of the valve orifices were aortic, 6.0 cm.; pulmonary, 5.5 cm.; mitral, 9.0 cm.; and tricuspid, 10.5 cm. The depth of the ventricles measured, from the base of the semilunar cusps to the apex of the chamber, left 8.5 cm. and right 10.0 cm. The left ventricular orifice of the interventricular septal defect was situated 1.5 cm. behind the edge of the subaortic muscular shelf and had a diameter of 0.4 cm. The right ventricular orifice was 0.5 cm. in height while the defect itself was about 1.0 cm. in length. The diamond-shaped area of congenital thinning in the conus measured 2 by 1.5 cm.

MICROSCOPIC DESCRIPTION

Wall of the Right Ventricle Opposite Interventricular Septal Defect.—Sections from this area disclosed numerous small excrescences on the endocardial surface, composed chiefly of masses of conglutinated platelets and large numbers of cocci occurring singly and in small clumps. Underneath these was a wide layer composed of erythrocytes, fibrin, polymorphonuclear leucocytes and mononuclear phagocytes, many of which were necrotic. The fibrin at the base of this zone filled the interstices between the thick collagenous fibers of the endocardium, which beyond the lateral limits of the thrombus consisted of a wide zone of compact relatively avascular scar tissue. Immediately underneath the area of thrombus formation the myocardium was replaced by young granulation tissue.

Area of Thrombus Formation in Descending Branch of Left Coronary Artery.—The deeper half of the vessel wall, lying in epicardial fat was surrounded and its adventitia and outer portion of its media destroyed, by a large abscess which showed two or three discrete areas of necrosis near the center and an area of granulation tissue about the periphery. The intima of the coronary artery in this situation was covered with thrombotic material. Several small localized inflammatory lesions and hemorrhages were noted in the neighborhood of the large abscess especially on the surface of the pericardium.

Left Aortic Cusp.—This was markedly thickened by old hyalinized connective tissue. About the midportion of the ventricular aspect of the cusp there was a large area of ulceration which extended through approximately three-quarters of the thickness of the cusp. The margins were undermined and the floor of the ulcer was covered by thrombotic material. The surface of the lesion contained dense colonies of cocci. The surrounding area was densely infiltrated with inflammatory cells, chiefly polymorphonuclear leucocytes which had coalesced in several places to form small abscesses, causing additional destruction of the unperforated portion of the cusp.

Endocardial Pocket.—This consisted of an endothelial-lined, cusplike, hyaline structure in which a few shrunken fibroblasts remained. Where it arose from the heart wall, the fibers composing the cusp ran parallel with the endocardial surface. In the valvular portion proper, however, the axis of these fibers changed abruptly so that they ran in a direction perpendicular to the endocardial surface except at the extreme tip where they terminated in a whorl formation.

Myocardium.—There were innumerable, scattered, small, focal inflammatory lesions composed of necrotic muscle fibers, mononuclear phagocytes, polymorphonuclear leucocytes, lymphocytes and plasma cells in about the proportion named. These lesions were distributed in relation to vascular structures. The small arteries were generally thickened and embedded in perivascular fibrous tissue; their walls were edematous and their lumens narrowed and sometimes obliterated. Several areas in the myocardium were replaced by dense scar tissue in which a few atrophic muscle fibers were present.

Aorta.—The arterial branches of the vasa vasora of the adventitia were frequently thickened. There were several small necrotic and granulating foci in the media and adventitia.

DISCUSSION

The anatomical basis for the defects present in this case appears to consist of an imperfect resolution of the bulbis cordis manifested by an incomplete and defective formation of the aortic septum associated with partial failure of spiral rotation. The bulbis cordis of the fetal heart is a muscular structure lined by spirally arranged endocardial swellings, the proximal and distal pairs of which are early recognizable. The situation of the former marks the division of the pulmonary conus from the right ventricle, while the latter corresponds to the site of the future semilunar cusps. Both unite with the descending septum aorticopulmonale to form the aortic septum which, when completed, divides the pulmonary artery from the aorta and also completes the division of the primitive heart by forming the upper and anterior interventricular septum (bulbar septum) in the region of the pulmonary conus. At one phase of the development the septum aorticopulmonale has descended from above while the distal and proximal bulbar swellings have fused in the midportion and below, thus temporarily permitting two openings to connect the two arterial trunks. The proximal opening lies below the semilunar valves and leads from the aortic into the pulmonary conus; its persistence in this case resulted in the interventricular septal defect. The distal opening in the aortic septum lies behind an aortic sinus (most frequently the right) and leads into the conus of the right ventricle between or beneath the pulmonary cusps. The homologue of this latter opening is normally found as the foramen Panizzae in the crocodile (Abbott[1]). In human beings, malformations in this region provide the embryologic basis for congenital lesions of the right aortic sinus of Valsalva. In our case the thinning of the bulbar aortic septum probably represented a defective formation of connective tissue obliterating the opening. Aneurysms with or without rupture (Abbott[2]) and a failure of the opening to become obliterated (Rickards[3], and Charteris[4]) are related lesions with an identical embryologic basis.

The dextroposition of the aorta can be accounted for on the basis that the aortic septum failed to undergo a complete spiral rotation. This dextroposition was of additional interest and significance in that it was responsible for two other features. The first of these concerns the right orifice of the bulboseptal defect which opened behind the septal cusp of the tricuspid valve. After examining the specimen Abbott[5] stated that were it not for the typical situation of the left ventricular ostium and for the associated congenital thinning of the wall of the right aortic sinus, this unusual situation of the right orifice would invalidate the classification of the defect as being bulboseptal. The second result of the dextroposition was the formation of a subaortic stenosis by a prominent muscular shoulder derived from the interventricular septum directly below the aortic orifice. That a stenosis existed in this region is further indicated by the presence of an endocardial pocket upon the subaortic muscular prominence. Although the congenital origin of these pockets has been suggested by Sotti,[6] the consensus of opinion (Kaewel,[7] and Ziegler[8]) is that they are acquired lesions due to mechanical or inflammatory factors. Depending upon the direction of their opening these struc-

tures have been designated by Krasso[9] as diastolic and, Saphir[10] especially, has emphasized their importance as indicating morphologic evidence of functional conditions within the heart.

CLINICAL-PATHOLOGIC CORRELATION AND SUMMARY

The clinical events which overhang a patient with congenital defects of this type are bacterial endocarditis and aneurysmal dilatation with rupture of the aortic sinus. The lesions of bacterial endocarditis tend to occur about the opening of the defect and secondarily upon the walls of the right ventricle where the repeated impaction of the shunted blood results in a localized area of lessened resistance. The aortic valve in this case was the seat of an old sclerosing lesion upon which an acute bacterial endocarditis was superimposed. The infected blood from the left ventricle was shunted through the bulboseptal defect into the chamber of the right ventricle where its impaction upon the outer wall resulted in extensive mural endocarditis; the infected thrombi in the ventricular chambers provided the source of embolic abscesses which were found in the organs of both the lesser and greater circulations. The only evident source of infection for the bacterial endocarditis was a slight nasal sinusitis which we regard as of doubtful importance. The hematuria was due to multiple infarcts of the kidneys while the headache and paralysis resulted from similar disturbances with abscess formation and hemorrhage in the right cerebral hemisphere with suppurative involvement of the meninges. The clinical evidence of areas of infarction involving the extremities provided the diagnostic clue to the acute thrombotic lesions within the chambers of the heart and embolic occlusion of arterial branches in the brain and kidneys. The presence of significant physical signs, the systolic thrill and harsh murmur heard best at the third left inner space and unassociated with cyanosis, led to the clinical diagnosis of interventricular septal defect. The indurated inflamed area about the left coronary artery coupled with the presence of thrombotic material in the lumen of the vessel was regarded at the autopsy table as probably indicating embolism of that vessel. A study of the microscopic sections from this area, however, made it apparent that an abscess in the epicardial fat was the primary lesion, the thrombosis of the vessel being a secondary feature.

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STUDIES ON ANTHRAX*

Clinical Report of Ten Human Cases

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THE cases of anthrax to be reported in this paper occurred in a Delaware County mill which handles goat hair imported from the Orient (China and India), for the manufacture of inner-lining. This mill has been in operation for over twenty years, and not until March, 1933, has there ever been reported a case of anthrax. Since then, four cases occurred among workers attached to the carding room, where the compressed bales of hair are taken apart and the hair is dusted out and carded. One case occurred in a woman who worked in the weaving room, handling hair bobbins. She was married to a man who had had anthrax and had recovered from it several months before. Two of the patients were children who lived in the mill-town. Their houses were located directly behind the carding room. The boys had never been within the mill's buildings; however, they played ball near the ground where waste hair from the carding machines was burned. Their mothers worked in the weaving department of the mill. The eighth case occurred in a Philadelphia plant which obtained its hair bobbins from the local mill by truck.

All the cases were studied in the Chester Hospital, and the clinical diagnosis of anthrax was confirmed in each case by smear and culture.

It is obvious that in spite of the present regulations governing the importation of hair, some infected material got to this mill. An attempt was made to determine the source of infection, and various samples of hair, taken at random, were studied bacteriologically by Dr. George Sickel of this city. Anthrax bacilli were recovered from one sample, on culture and guinea pig inoculation. Several months later the tests were repeated. Samples of dust and waste hair gotten from carding machines A and B, and a mixture of the same material obtained from the dust box, were examined bacteriologically.

On culture, a prolific overgrowth of contaminating organisms made the search for anthrax bacilli impossible. The samples were each placed in a glass funnel lined with gauze, and 100 c.c. of neutral plain bouillon were

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poured over it. The material filtered through was collected and injected into a series of guinea pigs. Only the animals injected with the dust box sample filtrate died of anthrax (Table I).

ANIMALS GUINEA PIGS	DOSE SUBCUT.	DATE	RESULTS
1	¼ c.c.	4/6/34	Dead 4/ 9/34 Positive anthrax
2	¼ c.c.	4/6/34	Dead 4/10/34 Positive anthrax
3	¼ c.c.	4/6/34	Dead 4/10/34 Positive anthrax
4	1 c.c.	4/6/34	Dead 4/ 8/34 Positive anthrax
5	1 c.c.	4/6/34	Dead 4/ 8/34 Positive anthrax
6	1 c.c.	4/6/34	Dead 4/ 8/34 Positive anthrax

Heart blood cultures of each guinea pig made on nutrient agar slants and on tubes of nutrient bouillon incubated twenty-four hours at 37.5° C. showed anthrax growth. Smears made from the cultures and examined microscopically revealed anthrax organisms.

The growth on agar slant was emulsified with 5 c.c. of normal saline solution. One cubic centimeter of this emulsion was then diluted with 40 c.c. of normal salt solution and 0.5 c.c. of this solution was injected into each of two rabbits (Table II).

RABBITS	DOSE SUBCUT.	DATE	RESULTS
1	0.5 c.c.	4/13/34	Dead 4/15/34 Positive anthrax
2	0.5 c.c.	4/13/34	Dead 4/16/34 Positive anthrax

Heart blood cultures and smears were made from each rabbit. They revealed anthrax organisms.

As it is seen from these results, the anthrax organism present in this mill and which caused the cases of anthrax to be reported in this paper was a very virulent strain.

We were also interested in determining whether the water, before and after use in the various buildings, became contaminated, since it drained into a nearby stream. Both on culture and animal inoculation (six guinea pigs were each injected subcutaneously with 5 c.c. of the water to be studied) the various water samples proved negative for anthrax. The tests were repeated twice, two weeks apart.

REPORT OF CASES

CASE 1.—A. B., a white male, eighteen years of age, was seen on March 25, 1933, when he stated that two days before he had scratched his face while at work in the carding room. A typical lesion, the size of a coat button, was found on the right upper neck. The indurated area of painless edema was over three inches in diameter. The lymph nodes were not palpable. On admission to the Chester Hospital, the temperature was 101° and pulse 90.

Following the local administration of serum given according to the technic of Regan,[1] there was marked spread of the swelling. On March 26 the face and neck were markedly edematous. At 1:00 P.M. a blood culture was taken and proved to be sterile. The edema spread to the chest, and by the following morning, it extended down to the costal margin. The skin showed a bluish mottling. The spleen was not palpable. The patient complained of tension and pressure within the swelling. On the twenty-seventh the face was distorted. The

lesion consisted of a black ulcer half a centimeter in diameter with a surrounding vesicular ring, and a zone of redness. The temperature dropped to normal, while the pulse rate was very rapid.

Dr. Lucchesi of the Philadelphia Municipal Hospital, who was called into consultation, advised the administration of a large dose of serum (300 c.c.). The same night the patient became restless and delirious. The skin of the neck split along the lines of cleavage. The temperature rose to 102.6°. The patient died on March 28, 1933, at 4:00 A.M., on the fifth day of the disease.

TABLE I

CASE 1

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
3/25/33	3:00 P.M.	85 c.c.	Intraven.	T. 103°	Swelling spread involving
		15 c.c.	Locally	P. 114	neck and jaw. W.B.C., 4,400; Polys, 62 per cent
	10:00 P.M.	50 c.c.	Intraven.	None	Further spread of edema
		15 c.c.	Locally		
3/26/33	9:00 A.M.	30 c.c.	Intraven.	T. 100°, P. 100	1:00 P.M. Blood culture sterile
	2:00 P.M.	50 c.c.	Intraven.	T. 100°, P. 100	
	11:00 P.M.	50 c.c.	Intraven.	T. 103°, P. 140	Neck, face, chest markedly swollen
3/27/33	10:00 A.M.	50 c.c.	Intraven.	T. 103°, P. 140	Patient toxic, pulse rapid, delirious
	8:00 P.M.	300 c.c.	Intraven.	T. 103°, P. 140	

An autopsy was performed by Dr. George Sickel six hours after death. The positive findings were as follows: Small black eschar was found on the right side of the neck just below the mandible. Extending from it over the lower face, neck, and chest to the costal margin, the subcutaneous tissues were the seat of a very marked and peculiar gelatinous edema. The chest wall was 6 to 7 cm. thick, and its cut surface resembled that of gelatin. A serous fluid ran out from the dependent portion of the incision. It was not hemorrhagic and did not contain gas. The cut vessels did not bleed, and in the subcutaneous tissue, they appeared as orange yellow projections as if they were thrombosed. The lungs did not show any pathologic change, except for an old healed primary tubercle. The heart was normal except for a few petechial spots on the anterior surface of the ventricles near the atrioventricular groove. The abdomen showed normal relations. The liver only showed loss of normal mottling. The spleen was but slightly enlarged and fairly firm. Its cut surface showed very prominent malpighian bodies. It did not resemble the soft splenic tumor of acute infection. The stomach showed a large ragged hole in the posterior wall at the cardiac end and just below the esophageal opening. There were no inflammatory reactions around this opening and surrounding peritoneum (probably due to postmortem digestion). The lymph glands were not enlarged. Blood obtained from the heart as well as smears from the spleen showed on culture anthrax bacilli. This organism was injected into a guinea pig by the subcutaneous abdominal route, with the production of a similar gelatinous swelling and death within forty-eight hours. Smears from the guinea pig's spleen showed anthrax bacilli.

Microscopic study showed the following:

Human Sections.—The heart showed cloudy swelling and edema; some capillaries were fairly full but not distended. The lung showed marked swelling of the interalveolar septa, caused largely by greatly distended capillaries plus mononuclear cell infiltration. The alveoli were distended, but showed no exudate. In some areas they contained a few red blood cells. The spleen did not show the engorgement seen in other acute infections. The malpighian bodies were enlarged and appeared to show an excessive number of large mononuclear cells. The pulp was fairly well filled with red blood cells and lymphocytes. The liver showed marked changes ranging from cloudy swelling to complete necrosis. The latter was widely and irregularly distributed, involving large areas. The capillaries were here and there, well filled with red cells, but in most areas the spaces between liver cords were filled with granular débris. In the kidneys, the glomerular tufts were well distended and in some areas the

intracapsular spaces contained a granular material. The tubular epithelium showed extensive degeneration and desquamation, with congestion of the intertubular capillaries. The subcutaneous tissue from the chest wall, at the level of the diaphragm, showed a very intense edema and cellular infiltration, mostly of polymorphonuclear cells, with a fair number of lymphocytes.

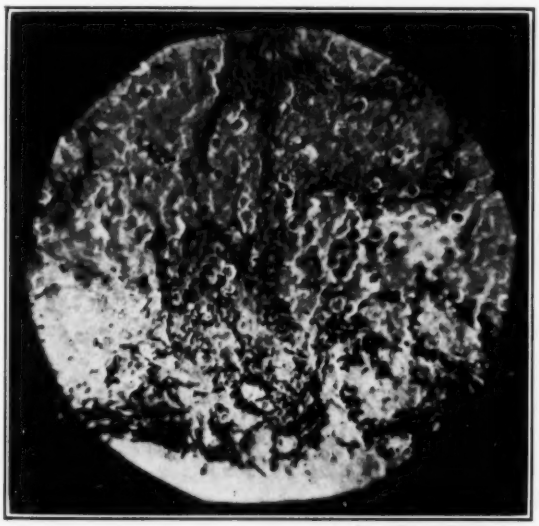

Fig. 1.—Section of guinea pig heart. High dry lens. Approximately 440 diameters magnification. Note great many clumps of anthrax bacilli in the blood clot attached to the endocardium. Many organisms are seen within and outside of blood vessels of the myocardium. (Lillie's stain.)

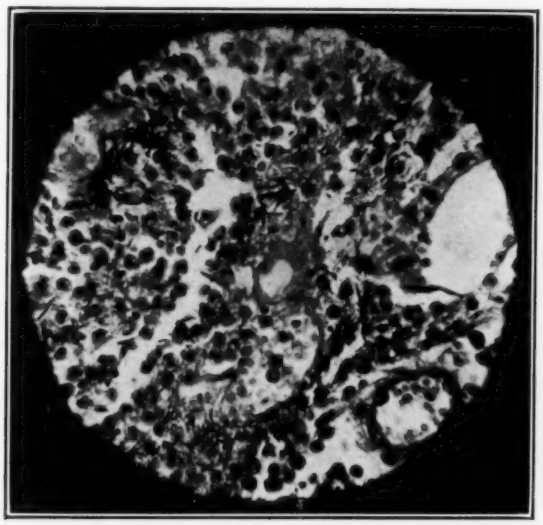

Fig. 2.—Section of subcutaneous tissue of chest wall of human case. High dry lens. Approximately 440 diameters magnification. Anthrax bacilli are shown in great abundance in tissue spaces. (Lillie's stain.)

Guinea Pig Sections.—All the organs showed the same type of reaction as described for the human sections, except that it was much more intense. The capillaries were congested throughout, being filled with red cells and anthrax bacilli, but they did not give one the impression of being blocked. Actually, the smaller arteries appeared more occluded than the

capillaries. While the guinea pig sections stained by hematoxylin-eosin showed anthrax bacilli, these are much better shown by Lillie's modification of Gram's stain.[7] Great masses of bacilli were seen in the heart's blood, lung, spleen, etc. (Fig. 1). In the human sections careful scrutiny detected anthrax bacilli in the subcutaneous tissue of the chest and the spleen. An occasional organism was found in the renal glomeruli (Figs. 2, 3, and 4). Although no

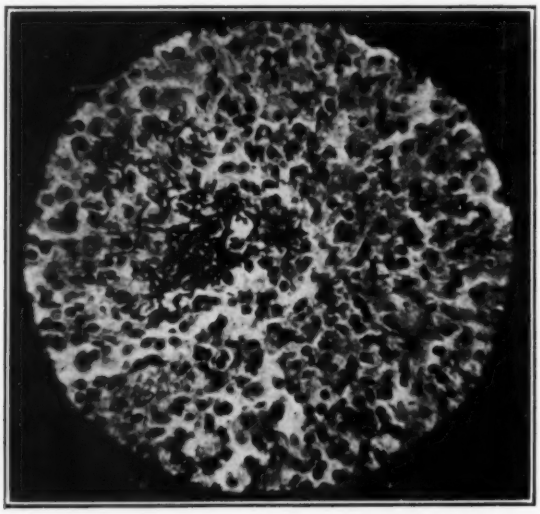

Fig. 3.—Section of human spleen. High dry lens. Approximately 440 diameters magnification. Note clump of bacteria. (Lillie's stain.)

Fig. 4.—Section of human kidney. Oil immersion view of glomerulus. Approximately 1080 diameters magnification. Note two anthrax bacilli partly superimposed on each other, in the right lower field. (Lillie's stain.)

serial sections were made, it was obvious from the study of our material, that death was not caused by the mechanical blockade of the capillary bed, as maintained by some pathologists. Even in the guinea pig, where the number of organisms found in the tissues was enormous, a great many of the blood vessels were patent, and even those that showed many organisms, were only partially filled.

COMMENT

Although within forty-eight hours after admission to the hospital the patient received 315 c.c. of serum intravenously, in small repeated doses, and 30 c.c. locally around the lesion, the course of the disease as measured by the edema and general intoxication was progressively for the worse. It is of interest to note that the spread of the edema occurred very soon after the local injections of serum, and though it was interpreted at first as a local reaction similar to that reported by other observers,[1, 2] it was later found at autopsy to be the result of a direct spread of the anthrax bacilli along the tissue spaces. It is our impression that the local injection of serum by mechanically separating the tissue spaces facilitated, rather than hindered, the spread of the organisms. Since a pronounced effect upon the local edema can be obtained by the intravenous injection of large doses of serum as evidenced by our other cases, we feel that the injection of serum around the lesion is of no particular advantage, and that it may actually do harm. Although in the light of our later experience the amount of serum given to this patient was small, it is well to remember that he was seen early in the disease, and that recoveries have been reported in similar or worse cases through the use of even smaller doses of serum.[3] It is remarkable that a large dose of serum given six hours after the blood was found to be sterile did not prevent the death of the patient ten hours later, when the organisms were recovered from his heart and spleen.

CASE 2.—T. S., a white male, aged twenty-four, was seen on the morning of May 19, 1933, when he stated that on the previous day, he had scratched his hand while cleaning a brush in the carding room. The left middle finger showed three fine linear scratches about 0.5 cm. long on the dorsal surface above the middle joint. Adjacent to the middle scratch, there was a small, red, flat painless papule, hard to the touch. Temperature and pulse were

TABLE II

CASE 2

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
5/20/33	1:00 A.M.	300 c.c.	Intraven.	Slight chill 102° fever		Temperature normal in A.M. W.B.C., 10,700.
	4:00 P.M.	200 c.c.	Intraven.	Slight chill T. 100.4		Polys, 80 per cent. Blood culture sterile
5/21/33	8:00 P.M.	50 c.c.	Intramusc.	Local pain		Patient feels well. Three painless axillary glands present
5/22/33	9:00 P.M.	50 c.c.	Intramusc.	Local pain		W.B.C., 7,500. Polys, 56 per cent
5/23/33	4:00 P.M.	100 c.c.	Intraven.	T. 100		Smear positive for anthrax
5/31/33	9:30 P.M.	40 c.c.	Intramusc.			Mild serum sickness since 5/25. Lesion had enlarged with a purple blue bleb at its margin
6/ 1/33	10:00 A.M.	100 c.c.	Intraven.	T. 99.6		Serum sickness aggravated. Bleb spreading. Smear positive for anthrax
6/ 2/33	10:00 P.M.				0.6 gm.	
6/ 3/33	10:00 A.M.	100 c.c.	Intraven.	T. 99.4	0.9 gm.	Mild serum sickness. Lesion enlarging
6/ 4/33	10:00 A.M.				0.9 gm.	Blood culture taken two days later sterile
6/10/33						Horse serum, 1-100, intracut. Negative. Horse serum 1-10, intracut. Slightly positive

normal. Six hours later he complained of headache and malaise. Temperature was 102° and pulse 100. The papule, still hard and red, had enlarged to the size of a dime. There was slight, nonpitting edema. The left epitrochlear and axillary nodes were palpable, the latter being painful. On incision, there exuded from the lesion a few drops of thick, yellow fluid from which anthrax bacilli were recovered.

The course in the hospital is outlined in Table II.

Notwithstanding the evidence of serum sickness, antianthrax serum, reinforced by neoarsphenamine, was given on June 1 because of the marked spread of the lesion, the appearance of a bluish red blister at its edge, the persistent adenopathy, and the continued presence of anthrax bacilli. No untoward reaction resulted. On June 7, 1933, the skin of the blister and the eschar were removed, leaving a shallow ulcer which healed completely in about two weeks.

CASE 3.—W. S., a white boy, ten years of age, was admitted to the Chester Hospital on the night of Sept. 10, 1933, with a diagnosis of anthrax of the face. The temperature was 102.4°, pulse 110. The lesion was a typical carbuncle in a rather advanced stage (third day). The right submaxillary triangle was swollen, inflamed and tender, with a red streak running across it to the sternoclavicular joint. No glands were felt. When we saw the patient in the morning of the eleventh, the edema had increased a little. Serum was given according to Table III.

On Sept. 26, 1933, the eschar was removed leaving a small punched out ulcer which healed in ten days leaving a small flat scar. On October 14, intracutaneous testing with horse serum

TABLE III

CASE 3

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
9/11/33	2:30 P.M.	450 c.c.	Intraven.	104° fever Slight chill		Temperature down to 99.2° in 6 hours. Blood culture sterile. Urine showed a heavy cloud of albumin
	11:45 P.M.	500 c.c.	Intraven.	104.4° fever Slight chill	0.45 gm.	Neck less swollen. Not tender
9/12/33	1:00 P.M.	200 c.c.	Intraven.		0.6 gm.	Swelling markedly decreased. Two small cervical glands on the right. W.B.C., 13,500. Polys, 86 per cent
9/13/33	11:00 A.M.	100 c.c.	Intraven.		0.6 gm.	Temperature normal. Hard swelling at angle of jaw due to lymph node. Lesion enlarging. Urine shows trace of albumin
9/14/33	5:00 P.M.	50 c.c.	Intramusc.	Local pain		Gland at angle of jaw size of walnut. Temperature normal
9/20/33	3:30 P.M.	50 c.c.	Intramusc.	Local pain		On 9/16/33, lesion negative for anthrax bacilli. Large node at angle of jaw hard, not tender. Few cervical glands on the right. Lesion dry with black eschar. W.B.C., 7,600. Polys, 63 per cent
9/29/33	Discharged from the hospital					Horse serum, 1-100, intracut. Test negative

Fig. 5.—Case 2. Lesion as it appeared on the third day of illness. Note the ulcer and the swelling of the joint.

Fig. 6.—Case 4. Lesion as it appeared on day of admission to the hospital. Note the ring of vesicles around the ulcer, and the marked edema of the forearm.

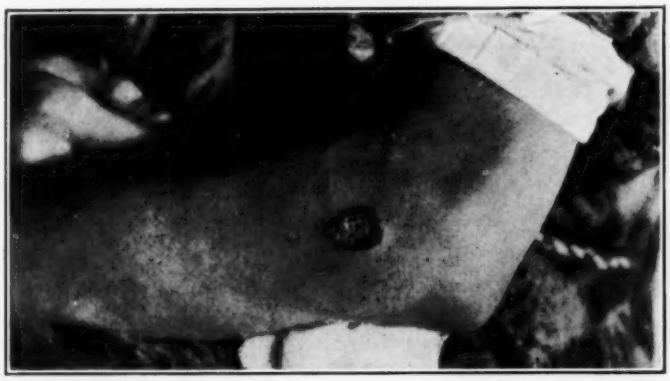

Fig. 7.—Case 4. Lesion as it appeared on day of discharge from the hospital after treatment. Note the epithelial islets at site of former carbuncle and the disappearance of the edema.

(1-10) was plus-minus. The patient had never had serum sickness in spite of the large repeated doses of antianthrax serum.

CASE 4.—W. D., a white male, eighteen years of age, was well until Oct. 6, 1933, when he scratched his left forearm while at work in a Philadelphia plant, which obtained its raw material (hair bobbins) from the Delaware County mill. Two days later he noted a small, painless, red pimple at the site of the untreated scratch. This was self-treated until the twelfth, when he was referred to us for diagnosis. Examination revealed a well-developed lesion with a central black eschar and four surrounding vesicles on the external surface of the left forearm below the elbow. The upper half of the forearm was tensely swollen and somewhat hot. The skin was slightly red. The left epitrochlear gland appeared as a visible tumor the size of a large walnut. Two axillary nodes were large and painful. There was a negative history for allergy, and the patient had never received any horse serum before. The patient was admitted to Chester Hospital on Oct. 12, 1933, at 1:45 P.M.

TABLE IV

CASE 4

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
10/12/33	8:00 P.M.	1000 c.c.	Intraven.	In the middle of the injection patient got hot and forehead itched. Toward the end, a few small hives and pruritus appeared, lasting 5 minutes. Slight chill. T. 99.8°		Throughout illness he had a very slight febrile response. Urine showed a cloud of albumin. W.B.C., 8,350. Polys, 81 per cent. Blood culture sterile
10/13/33					0.9 gm.	Few hives on face. Edema decreased
10/14/33	3:00 P.M.	100 c.c.	Intramusc.	T. 100° Local pain	0.9 gm.	Epitrochlear and axillary glands larger
10/15/33					0.9 gm.	
10/18/33	9:00 P.M.	100 c.c.	Intramusc.	Few hives and pruritus		Vesicles around the lesion dry. Smear positive for anthrax bacilli. Diffuse adenopathy
10/23/33						Eschar removed. Shallow ulcer. Healed in 2 weeks
10/28/33						Discharged from hospital. Diffuse adenopathy still present. Horse serum (1-10) intracut. Test slightly positive

A week after discharge the generalized adenopathy had subsided, except for the left epitrochlear gland which was still very prominent. It decreased in size very gradually and was both palpable and visible for more than six weeks. We were tempted to remove it to determine whether it harbored live anthrax bacilli, but we feared that surgical manipulation might activate this possible latent focus.

CASE 5.—A. K., a white male, forty years of age, was working in the carding room in the noon shift on Nov. 1, 1933, when he scratched the right side of the neck with a drum wire about 7:00 P.M. The next day when he reported to work at 2:00 P.M., a boil was noted at the site of the scratch, and he was referred to us for treatment. There was a "carbuncle" over the mesial border of the right sternomastoid muscle, with an area of painless edema three inches wide. Temperature and pulse were normal (Table V).

On November 13 the lesion began to show involutionary changes. The eschar was removed on the fifteenth, and the patient was discharged five days later. The ulcer was completely healed in about ten days.

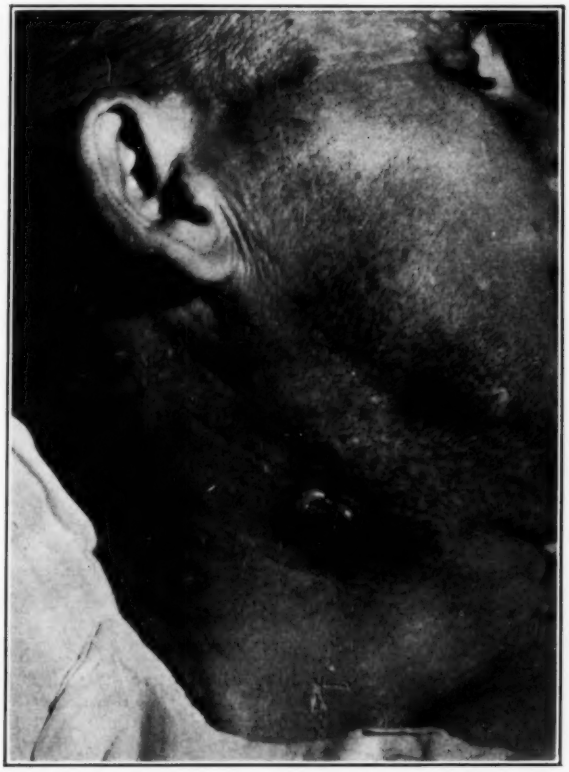

Fig. 8.—Case 5. Taken thirty-six hours after treatment was begun. All edema disappeared. Note collar of tense vesicles around central ulcer.

CASE 6.—H. G., a white boy, twelve years of age, lived in the village right at back of the carding room. He was seen on the evening of Dec. 12, 1933, when he gave the following history: On December 7 he noted a pimple on the right upper cheek. Two days later it looked the same, itched a little, but there was no pain or swelling. On the tenth, the sister scratched him above the pimple, drawing some blood. The pimple turned black while he was at school. When he returned home, his mother noted that the entire right cheek was swollen. He was feverish and complained of headache and pain in the upper right side of the neck. On examination, temperature was 100.4° and pulse 110. The right cheek was swollen and showed a carbuncle in its center, with a deep scratch 1½ cm. above it, but bearing no relation to it. The lesion had a black dry center. All the cervical glands were enlarged on the right. The boy had received diphtheria toxin-antitoxin in 1928.

On December 25 the patient was well, the ulcer had completely healed, and the adenopathy disappeared three days after discharge from the hospital.

TABLE V

CASE 5

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
11/2/33	7:00 P.M.	750 c.c.	Intraven.	Severe chill T. 100° Vomiting	0.9 gm.	Blood culture sterile
11/3/33					0.9 gm.	Swelling less marked. Ulcer enlarged. On its upper edge appeared a semilunar ring of tense dark vesicles
11/4/33						W.B.C., 9,600. Edema disappeared. Ulcer spreading
11/6/33	3:30 P.M.	100 c.c.	Intramusc.	T. 100° Local pain		Fluid from a blister showed a sparsity of cells, half of which were polys
11/8/33	9:00 P.M.	100 c.c.	Intramusc.	T. 100°		Occipital headache. Nuchal pain. No skin reaction. Joints and reflexes normal. Serum sickness? Meningitis? Smear negative for anthrax
11/9/33						Pruritus. Macular rash lasting 48 hours. Ulcer dry. Smear negative for anthrax

TABLE VI

CASE 6

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
12/12/33	9:30 P.M.	750 c.c.	Intraven.	Slight chill T. 100.2°	Blood culture sterile
12/13/33	3:30 P.M.	250 c.c.	Intraven.	Slight chill T. 102°	Swelling unchanged. Lesion enlarging. Glands are the same. W.B.C., 11,600. Polys, 85 per cent. Urine shows cloud of albumin
12/14/33					Temperature normal. Faint erythema. Swelling decreased. Lesion unchanged
12/16/33	9:00 P.M.	50 c.c.	Intramusc.	Slight pain	Large blister, the size of a nickel, containing clear fluid appeared at the lower edge of the wound
12/17/33					Mild serum sickness present. Swelling of the cheek still present. Blister turning black. Right upper neck swollen, due to enlarged glands
12/21/33					Severe serum sickness. Hives. Erythema. Chemosis and edema of the lids. T. 103°. Reaction lasted 3 days. Lesion dry, eschar removed. Smears taken on 12/19 and 12/23 negative for anthrax bacilli

Fig. 9.—Case 6. Taken before treatment on night of admission to hospital.

Fig. 10.—Case 6. Taken four days later. Note the superficial blister at the lower edge of the ulcer. This contained clear fluid.

CASE 7.—On Feb. 2, 1934, we saw a white man, S. P., aged twenty-four, who worked in the carding room of the mill. He had a lesion in the middle of the right calf the size of a quarter, with a dry black center, and a ring of tense flat red vesicles around it. The contour of the leg was obliterated by a tense and painfully tender swelling. The temperature was 100.4°. He had had tetanus antitoxin in 1930. He had apparently scratched his leg while at work. Smear and culture from the lesion were positive for anthrax bacilli.

On Feb. 2, 1934, at 6:00 P.M. the patient received 300 c.c. of serum intravenously, with no reaction. Twenty-four hours later the swelling had disappeared entirely. The blood culture was sterile; W.B.C. 8,000; polys 60 per cent. The lesion ran its natural course of peripheral vesiculation, drying of the vesicles, shrinking of the eschar and its separation, which occurred on the tenth. On the seventh the patient developed marked serum sickness which lasted four days. He was discharged from the hospital on February thirteenth, and he returned to work on March 5, 1934.

CASE 8.—A. K., a white female, forty-three years of age, had quit work in the weaving department of the mill, where she carried hair-bobbins, on Feb. 3, 1934. At a site of a scratch on the face, she developed a sore and a swelling which she treated herself without success. On February 7 we saw her at her home. She was found in the kitchen, her face bandaged in rags, and the examination was made under threat of calling the police. The left face was badly swollen, from the lower orbital ridge to the cervical crease below the jaw. In the center of the cheek, there was a very ugly black ulcer, larger than a twenty-five cent piece. It was surrounded by a ring of dark vesicles and a zone of erythema. The sore was painless. The gland at the angle of the jaw, the sublingual and the submaxillary nodes on the left side were swollen, hard, tender, and easily outlined. There was no cervical adenopathy. On admission to the Chester Hospital, the temperature was 99.4° and pulse 90.

TABLE VII

CASE 8

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
2/ 7/34	7:00 P.M.	300 c.c.	Intraven.	Slight chill Fever 103° Pulse 130	Malaise. Lesion larger, blisters oozing freely. Edema spreading around the jaw. Blood culture sterile. W.B.C., 8,500. Polys, 68 per cent
2/ 8/34	11:00 A.M.	300 c.c.	Intraven.	Slight chill Fever 103.2° Pulse 116	Temperature normal overnight. Edema spreading
2/ 9/34	10:30 A.M.	500 c.c.	Intraven.	No reaction	Temperature before injection 102°. Edema more marked. Lower lid swollen. Nasal outline distorted. Glands tender
2/10/34					Within 4 hours of the last injection edema decreased considerably, temperature and pulse returned to normal. Glands remained the same. Ugly black ulcer, larger than a half dollar. Peripheral blisters open, leaving a bright red edge around the lesion. Smear shows *Staphylococcus albus*
2/11/34	3:30 P.M.	100 c.c.	Intramusc.	No reaction	Edema completely gone. Glands decreasing in size, though still palpable. Nonpainful

On the thirteenth, the temperature went up to 100°, and skin manifestations of mild serum sickness appeared. The lesion was smaller, and the eschar was softening. Smear and culture still showed an occasional anthrax bacillus. The hives and erythema lasted thirty-six hours.

On the sixteenth, the eschar was removed, leaving an infected ulcer. Two days later, the patient was discharged from the hospital. She had been very uncooperative during her entire sickness, and a religious psychosis which was present before she contracted anthrax, made treatment very difficult. The glands had completely disappeared and the wound

Fig. 11.—Case 8. Taken on day of admission to the hospital. Note the marked edema of the entire left cheek and jaw.

Fig. 12.—Case 8. Taken after treatment. Note the shrunken eschar with dry edge, where vesicles were present. Observe total disappearance of edema.

was in the process of healing when she left the hospital on the eighteenth. We never saw her thereafter, but the husband reported that she was perfectly well and that her wound had healed in about two weeks.

CASE 9.—A. W., a white male, forty-eight years old, working in the card room, on Jan. 22, 1935, struck the right side of his face above the zygoma, with the loose end of a steel band, when he attempted to cut it, to open a compressed bale of hair. The scratch was immediately and thoroughly cauterized with pure phenol and painted with iodine. He continued to work, the wound being tightly covered with a sterile dressing. On Jan. 26, 1935, while at home in Camden, N. J., the face became swollen and a sore that turned black in twenty-four hours appeared at the site of the scratch. The patient did not report to my office until January 30, when the typical late lesion (eschar and vesicles) of cutaneous anthrax, plus extensive edema of the right side of the face with obliteration of the submaxillary space, were found. The submaxillary node was large, hard, and very tender, but the upper cervical nodes on the right were pealike. He was admitted to the Chester Hospital with a temperature of 99.6° and a pulse of 80.

On February 4, anthrax bacilli were recovered from the lesion. But two days later, smear and culture proved to be negative. On the fifth, the eschar had spread to the peripheral vesicles and a large swelling, the size of a small orange, was found behind the angle of the jaw. It was due to a lymph node. Temperature 99.4°. On the sixth, temperature 101°,

Fig. 13.—Case 9. Taken on day of admission to hospital. Note extensive edema present.

pruritus and crops of hives and erythema appeared, lasting forty-eight hours. The febrile reaction lasted for over a week. Although there was a diffuse, small adenopathy due to serum sickness, the right upper neck was tensely swollen because of large nodes. On the ninth, the eschar was removed, leaving an ulcer, the size of a nickel, with an infected base. On the fifteenth, the temperature dropped from 102° to normal. The nodes at angle and below the jaw were the size of walnuts, and firm to the touch. A pebble-like node was felt in front of the auditory meatus. All other nodes disappeared. Patient was sent home the next day. The submaxillary nodes decreased in size very slowly and were palpable for over two weeks. The ulcer gradually healed, leaving a very small scar.

CASE 10.—A. S., a white male, nineteen years old, who worked in the basement of the carding room (no other case had occurred there), was seen in my office, on Feb. 7, 1935. A small dry, yellowish, pealike papule, located in the center of a very bright erythematous area, the size of a quarter, was found on the right upper neck. There was neither edema nor cervical adenopathy. He complained of malaise and anorexia. The temperature was 100 and the pulse was 96. A smear obtained by rubbing an applicator, moistened with normal saline, against the papule, confirmed the diagnosis of anthrax.

On February 11, pruritus and urticaria appeared. Temperature was normal. On the fourteenth, there was marked serum sickness. Temperature rose to 102-104°, lasting four days. Patient also complained of migratory joint pains. On the eighteenth, patient was normal. Most of the eschar was removed, leaving an infected ulcer. There was no adenopathy. Patient was discharged on the twenty-second. Ulcer healed in two weeks.

TABLE VIII

CASE 9

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
1/30/35	4:45 P.M.	500 c.c.	Intraven.	None	Eye and skin tests to horse serum, neg. Blood culture, neg. Smear and culture from lesion, positive for anthrax
1/31/35	10:15 A.M.	500 c.c.	Intraven.	None	W.B.C., 8,750. Polys, 65 per cent. Face more edematous, with partial closure of eye. Swelling behind and below ear. Tenderness over submaxillary node disappeared 6 hr. after first injection; size unchanged. Lesion spreading
1/31/35	8:00 P.M.				Vesicles open. Swelling of face almost entirely gone. Lymph nodes showed no change

TABLE IX

CASE 10

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
2/ 7/35	3:30 P.M.	200 c.c.	Intraven.	None	Eye and skin tests, neg. for horse serum
2/ 8/35	10:30 A.M.	300 c.c.	Intraven.	Slight chill T. 103°	9:00 A.M. T. 101°. Lesion larger. Erythematous base is gone, but the center of the papule is turning black, with peripheral ring of fine, flat vesicles. Large area of tender edema has developed around the lesion. Upper anterior cervical nodes palpable
2/ 8/35	6:00 P.M.				Tenderness over edematous area gone. Swelling decreasing
2/10/35					Edema disappeared entirely. Smear and culture, negative for anthrax
2/12/35					W.B.C., 9,500. Polys, 84 per cent

COMMENT

The last two cases occurred within two weeks of each other, in different parts of the same building, a year after Case 8 had developed. During this anthrax-free period, no changes were made in the raw material employed, or in the processing to which it was subjected. The question arose, as to whether we were dealing with a recurrence of the old infection, or whether a new shipment of hair which had just arrived at the mill was responsible for the fresh outbreak. Tests similar to those described in the early part of this report, were made on samples of hair, obtained (1) from the center of a new bale, (2) from the surface of a new bale, (3) from a bale already opened and in the process of being carded, and (4) from sweepings of the storeroom floor, which contained dust as well as hair. The results were as follows: (1) Positive for anthrax. Three out of six guinea pigs died. (2) Negative for anthrax. (3) Negative for anthrax. (4) Positive for anthrax. Two out of six guinea pigs died.

These tests would indicate that in all probability the new outbreak of anthrax was caused by a recently imported lot of hair, which was contaminated.

It is of interest to note that in Case 9, thorough cauterization of the wound with pure phenol, immediately after the workingman was injured, did not kill the anthrax bacilli, although it may account for the rather long incubation period present. In Case 10 (which was seen very early, in the papule stage), the administration of 200 c.c. of serum intravenously did not prevent the occurrence of local edema and regional adenopathy. However, the latter was controlled by further administration of 300 c.c. of serum, indicating that a certain optimum dose is required in each individual case.

DISCUSSION

Antianthrax serum (human), manufactured by the Mulford Laboratories, Sharp and Dohme, was used in the treatment of all these cases. After the unfortunate experience with Case 1, we discontinued the use of serum locally, and depended entirely upon the intravenous route of administration. Occasionally, late in the disease, when slow absorption of the serum was desired, the intramuscular route was employed. Large quantities of serum were given slowly by the gravity method. The average total dose given in the recovered cases was 943.3 c.c. The smallest total dose was 300 c.c., while the largest was 1,350 c.c. of serum. The smallest single intravenous injection was 100 c.c., while the largest single injection of serum by vein was 1,000 c.c. Single doses of 400 to 500 c.c. of serum were used most frequently in our cases.

The anthrax infection, although localized to the skin, produces a systemic intoxication as evidenced by fever, rapid pulse, and malaise, which disappear under specific treatment. Our experience with these cases convinced us that an optimum dose of serum was necessary for each case, and that once that dose was given, definite results were to be expected. The temperature and pulse dropped sharply, while the edema showed a spectacular decrease and total disappearance within a short period of time. This local effect of the serum upon the edema must depend entirely upon the neutralization of some bacterial secreted factor, because neither the organisms themselves, nor the original adenopathy, showed any change after its administration. Thus anthrax bacilli were recovered from the lesions in Cases 2, 4, 8, and 9, very late in the disease, when the patients seemed to be well on the road to recovery. This persistence of the organisms and the marked regional adenopathy, which some observers consider as a dangerous possible source of reinfection[4] made us continue our specific treatment late in the disease. This appears, perhaps, unnecessary, in view of the fact that the glands remained enlarged for days and even weeks after the lesion was completely healed; yet the patients felt perfectly well, and made an uneventful recovery. In Case 9, enough serum was given to control the edema; the local lesion and the adenopathy received no further treatment, yet they underwent complete involution with restoration to normal. We are unable to state whether the glandular enlargement is due to actual invasion of the nodes by the anthrax bacilli, or whether they merely represent an inflammatory reaction to the products of the bacilli, which are themselves localized in the carbuncle. Serum does eliminate the pain which is sometimes present in the enlarged glands.

The course and rate of development of the carbuncle, however, appear to be unaffected by the administration of serum.

The antianthrax serum used in these cases did not produce any severe reaction, and the serum sickness which it induced in some of our patients was rather mild. The previous administration of horse serum somewhat aggravated the latter. This absence of severe reaction is particularly noteworthy in view of the tremendous doses of horse serum used intravenously in our cases. Because of this finding, we administered the serum, in the presence of serum sickness, without running into any difficulties.

The use of neoarsphenamine in large doses, which has been advocated by several European workers,[5] was in our experience of no value. When given in addition to the serum, it did not appear to alter or shorten the course of the disease, and its use was discontinued in our last five patients.

The leucocyte response in our patients was not very significant. It was normal in most of them, with leucopenia and slight leucocytosis in a few. After administration of serum, no noteworthy leucocyte response occurred, hence we feel that the modus operandi of specific serotherapy does not depend upon the mobilization of the white blood cells, as claimed by some.[6]

It is interesting to speculate on the nature of the processes of recovery in those infected with anthrax, and on the possible immunity conferred by an attack of this disease. Facts bearing upon this phase of the problem, as well as results obtained during attempts at active immunization of human beings by means of intracutaneous and subcutaneous administration of an anthrax antigen, will be reported in a separate communication.

It is obvious that treatment of the wounds, even when done early, is of no value in the prevention of anthrax. The hope for prophylaxis lies in the enforcement of strict laws governing the importation of hair from areas where anthrax is endemic; the possible disinfection of all hair at ports of entry under government control, as it is done in England; and the development of suitable means of active immunization against this disease.

SUMMARY

1. Ten cases of anthrax are here reported. Two patients were children, and one case was a woman.

2. Seven of the patients contracted anthrax while working in a mill, which uses imported goat hair for its raw material. The two children lived in the village surrounding the mill, immediately behind the carding room which was the center of infection. The other case occurred in a distant plant where the infection was carried from the local mill, via shipping cases or hair bobbins.

3. All patients but the first one, recovered.

4. Antianthrax serum was used intravenously in large doses in the patients who recovered.

5. Injection of the serum locally, around the anthrax lesion, was discontinued after the first case, because of the possible dangerous spread of the infection through the tissue spaces. This may be brought about by the mechanical separation of the tissues.

6. Neoarsphenamine was not found to be of any value in the few cases where it was given in addition to serum.

7. Studies to determine the source of infection, are given. Tests indicate that a rather virulent strain was responsible for the cases of anthrax reported.

8. Autopsy findings are given on the one death of our series.

I wish to express my gratitude to Drs. John Reichel and J. E. Schneider, of Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa., for their kind interest and valuable advice given during this study.

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THE INCIDENCE OF NONDIABETIC GLYCOSURIA*

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PHYSICIANS generally recognize that a small percentage of persons showing glucose in successive urine specimens do not have diabetes mellitus, that the condition is renal hereditary glycosuria.[1] Many are cognizant of the existence of another type, usually intermittent, of glycosuria, best described as nondiabetic glycosuria.[2] Careful search of the literature disclosed a single statistical investigation of the subject: Joslin[3] states that one-sixth of the persons referred to him on account of glycosuria did not have diabetes. Several physiologic experiments[4] point to the existence of this type of glycosuria, which none the less seems to have been generally overlooked in clinical literature. Isolated case reports[5] of persons with benign glycosuria, some of them mistakenly treated for diabetes for years,[6] appear infrequently. Several classifications[7] of the glycosurias have been proposed; all seem unsatisfactory for reasons which need not be discussed here.[8] The most satisfactory classification of the glycosurias would seem as follows: diabetic and nondiabetic.

It is my purpose to present a statistical analysis of 238 persons referred to the Diabetic Division of the Aaron Waldheim Health Clinic during the last six years with a tentative diagnosis of diabetes or on account of glycosuria

*From the Diabetic Division of the Aaron Waldheim Health Clinic and the Jewish Hospital.
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demonstrated in one or several urine specimens. Thirty-two (13 per cent) had nondiabetic glycosuria. Table I based on the glucose tolerance curve[9] summarizes these cases. Repeated tolerance tests were used as a check on the diagnosis. In no case diagnosed as nondiabetic glycosuria has it been necessary later to make a diagnosis of diabetic glycosuria; occasional patients at first considered to have diabetic glycosuria have later been considered nondiabetic.

CASE REPORT

A single case report, atypical in many respects, yet illustrative of the fact that hyperglycemia during the tolerance test does not of necessity mean diabetes, is given. This thirty-year-old white female first came to the clinic in 1928 on account of a duodenal ulcer which has since been quiescent under medical management. During hospitalization in 1929 a routine glucose tolerance test was done. In May, 1930, the patient first reported to the diabetic division because of pruritis vulvae; there have been no other complaints suggestive of diabetes. The tolerance curves are shown in Table I.

TABLE I

TRUE BLOOD SUGAR IN MG. PER CENT BEFORE AND AFTER 100 GRAMS GLUCOSE

DATE	FASTING	1	2	3	4 HOURS	BODY WEIGHT POUNDS
May, 1929	112	299	180	126		130
May, 1930	117	231		58		152
June, 1931	76	270		177		121
Oct., 1931	113	194	156	135	75	130
June, 1934	120	252	219	163		140
Dec., 1934	116	252	120	90		132
Oct., 1935	116	194	123	100	80	132

Of 62 urinalyses during this interval 28 showed sugar in varying amounts, 34 no sugar. Glycosuria occurred without regard to blood sugar level. The absolute dissimilarity of the curves should be noted. Had this patient been seen for the first time in June, 1931, or June, 1934, a diagnosis of diabetes would have been made—to be shown wrong in December, 1934. This unusual case has been presented instead of one more typical of nondiabetic glycosuria because it illustrates well a type of patient in whom I think antidiabetic therapy is not indicated.

DISCUSSION

To discuss the mechanism of glycosuria would carry us far afield and lead to no useful result.[10] I[11] am of the opinion that there is no relationship between the level of sugar in the blood and its appearance in the urine. My interpretation of the tolerance curve is based upon the relationship existing between a normal or nearly normal[12] fasting blood sugar level and the blood sugar level three hours after the ingestion of 100 gm. of glucose. If the latter is below 100 mg. per cent a diagnosis of nondiabetic glycosuria (should sugar appear in the urine) may unhesitatingly be made; if above 150 mg. per cent, a diagnosis of diabetes is almost always certain (regardless of the appearance of sugar in the urine); if the three-hour blood sugar level is between 100 and

TABLE II

SUMMARY OF THE PATIENTS SHOWING NONDIABETIC GLYCOSURIA

CASE	SEX	AGE	KNOWN DURATION (YR.)	YEARS OF OBSERVATION HERE	NUMBER OF GLUCOSE TOLERANCE TESTS	HIGHEST BLOOD SUGAR DURING TOLERANCE TESTS		LOWEST VENOUS BLOOD SUGAR LEVEL WITH AND WITHOUT GLYCOSURIA	GLYCOSURIA	WEIGHT	COMMENTS
						VENOUS	ARTERIAL				
1	F	19	14	7	2	76-113	103-161	54	In*	Under	Blood sugar of 42 with glycosuria and hypoglycemic attacks
2	F	20	3½	3	2	114-161	149	114	In	Norm	Treated elsewhere with insulin and diet on account of pruritis due to scabies
3	M	24	14	2	3	98-140	167	95	In	Norm	Treated elsewhere with insulin and diet
4	M	31	mo.	1	1	178	202	84	Tr	Under	Accidentally observed glycosuria
5	M	33	mo.	mo.	1	93		58	Tr	Norm	
6	F	35	3	3	3	129-146		128	R	Norm	Syphilitic
7	F	37	5	5	5	211-279	249-289	104	In	Norm	Duodenal ulcer and gallbladder disease
8	F	40	2	2	3	290-307			Oc	Norm	After loss of 24 pounds, tolerance curve assumed nondiabetic type. Symptom-free
9	M	42	18	3	2	93-96		89	R	Norm	Treated elsewhere for diabetes
10	F	42	2	4	1	201			R	Over	
11	M	42	mo.	mo.	2	175-201			In	Norm	Glycosuria noted after 20-pound box fell on foot
12	F	42	½	2	1	213		157	R	Under	
13	M	44	5	5	2	162-167	203	85	In	Over	Pathologic gallbladder on x-ray
14	M	45	1	1	1	78		78		Over	
15	M	47	4	4	1	158	182	121	In	Norm	Glycosuria when patient worried over loss of job
16	F	40	2	2	3	290-307		120	R	Norm	After loss of 20 pounds, tolerance curve assumed nondiabetic type; formerly diabetic
17	M	50	17	4	5	176-217		95	In	Norm	Treated elsewhere for diabetes
18	M	50	3	mo.	1	266				Over	
19	F	52	5	5	3	176-187	207		R	Norm	
20	F	54	4	4	6	126-262	245-266	187	R	Norm	Duodenal ulcer
21	M	55	13	5	2	108	182	84	Oc	Norm	Herniotomy without complication

TABLE II—CONT'D

CASE	SEX	AGE	KNOWN DURATION (YR.)	YEARS OF OBSERVATION HERE	NUMBER OF GLUCOSE TOLERANCE TESTS	HIGHEST BLOOD SUGAR DURING TOLERANCE TESTS		LOWEST VENOUS BLOOD SUGAR LEVEL WITH AND WITHOUT GLYCOSURIA	GLYCOSURIA	WEIGHT	COMMENTS
						VENOUS	ARTERIAL				
22	F	57	½	½	1	271			In	Over	
23	F	59	3	3	3	225-265			In	Under	First fasting blood sugar of 224 mg. per cent
24	M	57	2	2	3	207-330			R	Norm	Previously diagnosed diabetic here when somewhat overweight
25	M	59	5	5	3	168-192	177	89	In	Norm	
26	M	60	4	4	3	264-340	406	128	In	Norm	First curve typically diabetic
27	F	60	5	mo.	1	251				Over	Treated elsewhere for diabetes
28	M	63	mo.	mo.	1	210			In	Norm	
29	M	68	mo.	mo.	1	173			In	Norm	Prostatectomy without complication
30	F	68	3	3	3				In	Over	
31	M	71	4	4	3	128-194	208	115	In	Over	
32	M	74	4	4	3	116-175		116	In	Over	

Comment: Table II clearly shows: (1) the difference in the height of the venous blood sugar curve on repetition of the tolerance test; (2) an obvious lack of relationship between the venous and the arterial (capillary) blood sugar levels; (3) the irregular occurrence of glycosuria in these patients. More important, the column headed "Highest Venous Blood Sugar Level With and Without Glycosuria" emphasizes the absolute absence of a constant blood sugar level at which glycosuria occurs; it demonstrates that glycosuria occurs without regard to the blood sugar level; hence that glycosuria alone is of no clinical significance. Cases 8, 16, 23, 24, and 26 show the value of repeated testing if one wishes to avoid treating nondiabetic persons for diabetes.

*In, inconstant; Tr, transient; R, rare; Oc, occasional; Under, underweight; Norm, normal weight; Over, overweight.

150 mg. per cent, discrimination is necessary to make the correct diagnosis. Frequently in the latter case repetition of the curve, after the obese patient's weight has been reduced, is necessary. I have a very strong feeling that until the patient can be indubitably said to have diabetes it is unfair to place him upon an antidiabetic regime; in case of doubt it cannot conceivably do any harm to keep him under observation until a definite diagnosis can be made.

CONCLUSIONS

1. Glycosuria, differentiated by the glucose tolerance test, is of two types: diabetic and nondiabetic.

2. Over 13 per cent of persons showing glucose in one or more specimens of urine do not have diabetes. Thirty-two of 238 persons referred to the Diabetic Division of the Aaron Waldheim Health Clinic had nondiabetic glycosuria.

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A CHEMICAL STUDY OF THE ALUM-DIPHTHERIA TOXOID PRECIPITATE*

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HAVENS and Wells[1] showed in 1933 that diphtheria toxoid precipitated by an alum solution, the precipitate washed once with saline and then suspended in fresh saline, gave a product which in one inoculation immunized practically all of the children so treated. Prior to this Park and Schroder[2] obtained results in guinea pigs and children superior to those with ordinary toxoid, by using a toxoid containing 0.2 per cent alum, partially precipitated and not purified.

A detailed study of the conditions for preparing this diphtheria toxoid-alum precipitate as developed in this laboratory, and some of the properties of the precipitate are presented in this paper.

The diphtheria toxoid used was made from bacto-veal[3] broth and fresh veal broth by Dr. O. R. Povitzky, to whom we wish to express our thanks. The different lots of this material averaging about 150 litres each were given a preliminary test to determine the conditions for precipitation that would yield the best obtainable product since they showed such markedly diverse reactions upon the addition of alum. This method takes into account the factors of temperature, concentration of alum, and inability to precipitate some lots of the toxoid by the addition of alum alone until sodium chloride has been added. A 10 per cent solution of potassium aluminum sulphate, $K_2Al_2(SO_4)_4.24H_2O$, analytical reagent quality, was used.

A series of 108 test tubes, each having a capacity of about 8 c.c., was set up in 9 racks, each rack containing 12 tubes in 2 rows. To each tube, 5 c.c. of the toxoid being tested were added. The racks were segregated into 3 sets. One set was heated in a water-bath at 60° C. for 15 minutes and varying quantities of the 10 per cent alum solution were added to each row of tubes to give a final concentration of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 per cent alum. The tubes were shaken and heated for an additional 15 minute period. Solid sodium chloride was then added so that for a given concentration of alum in toxoid there would be 1, 2, 3, 4, and 5 per cent salt, and one as a control without salt. The tubes were shaken to dissolve the salt and bring down the precipitate. They were left at room temperature and read twenty-four hours later.

The other two sets of tubes were treated in the same way with the exception of the temperature factor. They were heated in a water-bath to 40° C. prior to the addition of the alum and salt. One set was put in the incubator at 37.5° C. until the following morning, while the other was left at room

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temperature. The amounts and appearances (granular, flocculent, etc.) of the precipitates and the appearances of the supernatants (clear, cloudy) were noted after twenty-four hours.

Two tables are presented giving the results of such preliminary tests, Table I for a toxoid prepared with bacto-veal broth, and Table II for a toxoid prepared with fresh veal broth. The "bacto-veal" toxoid gave the most satisfactory precipitates only on the addition of 4 per cent or 5 per cent sodium chloride and with 1 per cent or 1.5 per cent alum. Only in these cases were the supernatants clear. With the "fresh veal" toxoid (Table II) on the other hand, the most satisfactory precipitates were obtained with 2 per cent or 2.5 per cent alum and no added salt. In neither table were the 60° C. precipitates as satisfactory as those obtained at the lower temperatures.

These two tables are typical of the results obtained with the various toxoid preparations.

Final Test.—From the tables of the initial test, 4 or 5 tubes were chosen showing the greatest amount of precipitate, finely divided rather than granular, having the clearest supernatant fluid. For the toxoids shown in Tables I and II, the preparations marked with an asterisk were selected. Using the same concentrations of alum, and salt or no salt as indicated, 100 c.c. portions of the toxoid were precipitated under the same conditions to yield sufficient material for quantitative tests. The suspensions were allowed to settle for four hours, the supernatant fluids drawn off and the precipitates washed with saline equivalent to the amount of fluid removed. The following morning the washings were drawn off and enough saline added to make the suspensions up to the original volumes of toxoid. The suspensions were dissolved by the addition of 1 per cent solid sodium citrate and warming in water-bath at 40° C., and tested for flocculating units (Lf),[4] total nitrogen by the Kjeldahl method, and aluminum content.

The method for the determination of aluminum was adapted for use on diphtheria toxoid-alum precipitate by Clifford George Pope[5] and is most reliable where quantities of material are tested containing 0.5 to 1.0 mg. of aluminum. The toxoid-alum suspension was measured out, dissolved in 5 per cent borax solution, and the aluminum precipitated quantitatively with 8-hydroxy quinoline. The aluminum-quinoline precipitate was dissolved in hot hydrochloric acid (1:3) and the contained aluminum estimated by titration with excess standard potassium bromate-bromide and back titrated with standard sodium thiosulphate solution as in the Koppeschaar method.

The data given in Table III refer to the toxoid preparations for which the results of the preliminary tests were presented in Tables I and II.

The flocculation determinations were carried out in Dr. Olga R. Povitzky's division by Miss Minnie Eisner to whom thanks are due. So far as the character of the precipitate is concerned, a finely dispersed material is most desirable so that a uniform mixture for injection can readily be obtained. For the toxoids shown in Table III, the "veal" toxoid precipitate was well dispersed and slowly settling, while the "bacto-veal" toxoid precipitate was coarser in character and settled more rapidly. This difference was not always

TABLE I

COMPARATIVE STUDY OF FORMATION OF DIPHTHERIA TOXOID ALUM PRECIPITATE WITH VARYING QUANTITIES OF ALUM AND SODIUM CHLORIDE AT DIFFERENT TEMPERATURES (BACTO-VEAL)

ALUM		CONTROL			1% NaCl			2% NaCl			3% NaCl			4% NaCl			5% NaCl		
		INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT
0.5%	Supernatant																		
	Precipitate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1.0%	Supernatant	++			+	++		±			−	−		−	−	++	−	−	++|
	Precipitate	++	−	−	+++	+	−	+++	−	−	+++	+++	−	++++	++++	+	++++*	++++*	++
1.5%	Supernatant	++		++	±	++	++	−	±	++	−	−	+	−	−	±	−	−	−
	Precipitate	+	−	+	++	+	+	++	++	++	+++	+++|	+++	++++	++++	+++|	++++*	++++*	+++|*
2.0%	Supernatant			++			++	±	++	++	−	±	+|	−	±	+|	−	−	±
	Precipitate	−	−	+	−	−	++	+	±	++	++	+	+++	+++	+	+++	+++	++	+++
2.5%	Supernatant									++			++	+		+|	−	+	±
	Precipitate	−	−	−	−	−	−	−	−	+	−	−	++	±	−	++	+	+	++
3.0%	Supernatant																		
	Precipitate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Supernatant: ++++ (maximum cloudiness) → − (clear)

Precipitate: ++++ (maximum) → − (none)

TABLE II

COMPARATIVE STUDY OF FORMATION OF DIPHTHERIA TOXOID ALUM PRECIPITATE WITH VARYING QUANTITIES OF ALUM AND SODIUM CHLORIDE AT DIFFERENT TEMPERATURES (VEAL)

ALUM		CONTROL			1% NaCl			2% NaCl			3% NaCl			4% NaCl			5% NaCl		
		INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT
0.5%	Supernatant	±	±	+	±		+	−		−	−	−	−	−	−	−	−	−	−
	Precipitate		−	−						±	±	±	±	±	±	±	±	±	±
1.0%	Supernatant	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	Precipitate	±	+	++	±	+	+	±	+	+	±	+	+	±	+	+	±	±	+
1.5%	Supernatant	+	+	+		±	+	+	+	+	+	+	+		+	+	±	+	+
	Precipitate	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	+++	+++	++++
2.0%	Supernatant	−		−	−		−	−	−	−	−	−	−	−	−	−	−	−	−
	Precipitate	++++*	++++*	++++	++++	++++	+++	++++	++++	+++	+++	+++	+++	++++	+++	++++	++++	+++	++++
2.5%	Supernatant	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Precipitate	+++*	+++*	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
3.0%	Supernatant			−			−			−			−			−			−
	Precipitate	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Supernatant: ++++ (maximum cloudiness) → − (clear)

Precipitate: ++++ (maximum) → − (none)

found; that is to say the "veal" toxoid precipitate was not always more satisfactory so far as dispersion and settling were concerned. The individual preparations, whether from "veal" or "bacto-veal," varied without any apparent regularity in these properties.

The analytical data and calculations shown in Table III require little elaboration. The Lf per mg. N serves as an index for the satisfactory conversion of the diphtheria toxoid into the alum-toxoid precipitate, the greater the Lf and the smaller the N content per c.c., presumably the more satisfactory

TABLE III

FINAL TEST

SOLUTION TESTED	LF/C.C.	MG.N/C.C.	LF/MG.N	MG.AL/C.C.	MG.AL/MG.N	LF/MG.AL
Diphtheria Toxoid, Preparation No. 41 (Bacto-Veal)						
Original toxoid (bacto-veal)	10.3	4.41	2.3	0.00	0.00	0
Toxoid, 1% alum, 5% NaCl at room temp.	9.8	0.78	12.5	0.32	0.41	31
Toxoid, 1% alum, 5% NaCl in incubator	9.8	0.80	12.2	0.30	0.37	33
Toxoid, 1.5% alum, 5% NaCl at room temp.	10.1	0.69	14.6	0.24	0.35	42
Toxoid, 1.5% alum, 5% NaCl in incubator	10.1	0.81	12.4	0.23	0.28	44
Toxoid, 1.5% alum, 5% NaCl at 60° C.	8.75	0.74	11.8	0.26	0.35	34
Diphtheria Toxoid, Experimental No. 23 (Veal)						
Original toxoid (veal)	28 -28.3	(4.40)	(6.3-6.4)	0.00	0.00	0
Toxoid, 2% alum at room temp.	17.8-19	0.96	18.5-19.8	0.68	0.70	27
Toxoid, 2.5% alum at room temp.	16.6-19	0.78	21.2-24.3	0.71	0.91	25
Toxoid, 2% alum in incubator	17.8	1.22	14.5	0.75	0.61	23
Toxoid, 2.5% alum in incubator	16.6	0.79	21.0	0.74	0.93	22

the material for immunizing purposes. The Al contents and the ratios given in the table are of interest. From the data given here, the most satisfactory conditions for processing the bulks of the toxoids shown in Tables I to III would be for the "veal" toxoid 2.5 per cent alum at room temperature, and for the "bacto-veal" toxoid 1.5 per cent alum, 5 per cent sodium chloride, and room temperature.

Employing the same conditions giving the best results shown in the final test in any given case, the alum-toxoid precipitate is prepared in large graduated bottles. In general, the following procedure is used. The toxoid is heated in a water-bath to 40° C., then taken out and the required amount, as shown by the tests, of sterile 10 per cent alum and in some cases sodium chloride is added. The bottle is shaken until the precipitate sediments completely. The supernatant fluid is siphoned off four hours later, replaced by sterile saline, and shaken. This is left to settle overnight. The following morning the washings are siphoned off and enough sterile saline is added to give the original volume of toxoid used. One to ten thousand merthiolate is added as a preservative. Throughout this procedure sterile technic is used in the addition of sterile saline and alum and in siphoning off the supernatant fluids to avoid contamination.

As a result of studying a considerable number of toxoid materials prepared here, the tests necessary for determining the most satisfactory conditions for the preparation in bulk of the toxoid-alum precipitate have been simplified. Whether such simplification applies to preparations elsewhere, cannot be stated at present. In general, the preliminary tests need be carried out only at room and at incubator temperatures for "veal" toxoids; with alum concentrations between 1.5 per cent and 2.5 per cent and no added sodium chloride; with alum concentrations between 1.0 per cent and 2.0 per cent and no added sodium chloride and with 5 per cent added sodium chloride for "bacto-veal" toxoids. The most satisfactory precipitates obtained in these tests can then be used for the final tests, and the best of these last used for bulk preparation.

A number of diphtheria toxoid alum precipitate preparations were analyzed. It would lead too far to present the detailed results. A general summary will therefore be given.

Nitrogen contents:	
Original "Veal" and "Bacto-Veal" Toxoids	4.20 – 4.50 mg.N per c.c.
Toxoid alum precipitate, veal	0.90 – 1.41
Toxoid alum precipitate, bacto-veal	0.31 – 0.62
Aluminum contents:	
Toxoid alum precipitate, veal	0.61 – 0.76 mg.Al per c.c.
Toxoid alum precipitate, bacto-veal	0.23 – 0.32

In every case, the volume of the toxoid alum precipitate was the same as that of the original toxoid material. The nitrogen and aluminum contents of the "veal" toxoid precipitate materials averaged two or more times as great as that of the "bacto-veal" toxoid precipitate materials. Whether these relations have any practical significance for immunizing purposes cannot be stated. The biologic potencies (Lf per c.c. or Lf per mg. N or Lf per mg. Al) naturally depend upon the original potency of the toxoid. In general terms the loss in the precipitation was 20 per cent to 40 per cent, independent of the original value.

Only in very few instances over a period of two years was it found necessary to add salt to large lots of diphtheria toxoid to precipitate with alum. Some of the toxoids made of bacto-veal were the only ones which showed such a need for salt, because the addition of alum alone failed to yield visible results. On the whole, the toxoids made with fresh veal gave a more uniform reaction to the addition of alum. After using the fresh veal toxoid for a time, to the exclusion of the bacto-veal, more uniform results have been obtained with regard to fineness of texture, lighter color, and much more uniformly dispersed precipitate.

CONCLUSIONS

As a result of the many tests on diphtheria toxoid precipitation, the following conclusions may be drawn:

1. Diphtheria toxoid made with fresh veal broth gave a better product as far as color, fineness, and uniform dispersion of the precipitate was concerned.

2. In general, 2 per cent alum concentration was needed for the maximum precipitation of fresh veal toxoids, while 1.5 per cent alum concentration gave the maximum precipitation with the bacto-veal.

3. If the toxoid did not precipitate by the addition of alum alone, the addition of solid sodium chloride brought about the precipitation.

4. A better product was obtained when the toxoid was heated to 40° C. prior to precipitation.

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THE MORPHOLOGIC SUGAR METABOLISM IN THE HUMAN LEUCOCYTE CULTURE*

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THE morphologic representation of the sugar metabolism is only possible by the observation of the glycogen within the cells. The other products of carbohydrate metabolism do not give a specific histologic reaction. Thus, a complete representation of the whole carbohydrate metabolism cannot be brought about by histologic methods. Nevertheless the representation of the glycogen is sufficient for establishing differences of carbohydrate metabolism under different research conditions. The histologic glycogen reaction is a very sure one since Best has given us his new method.

The sugar metabolism in terms of changes in glycogen storage in the different cells has been presented by several authors. Pflüger, especially, has given us a distinct description of the connection between glycogen and administered carbohydrates. But all these researches have been made only on the liver cells of the living organism. The living organism is subject to very different influences which cannot be governed by us in our researches. We have tried, therefore, to examine the sugar metabolism according to the changes in the glycogen deposition in the human leucocyte. At first, we wished to study the glycogen deposition in the normal leucocyte culture and then after administration of different carbohydrates. Finally, we have examined the changes of the glycogen metabolism after addition of the different substances which govern the sugar metabolism, such as insulin. Under these circumstances, we hoped to obtain a complete picture of the carbohydrate metabolism in the human leucocyte culture. The observations have been made daily.

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The technical method of preparing the leucocyte cultures is described by us in a former work on the metabolism of the vital dyes in the human leucocyte culture. The demonstration of glycogen in the culture has been effected in that the whole culture was covered by a fine layer of collodion after fixation with alcohol, so that the glycogen itself could not be dissolved in water. Then we have proceeded in the manner described by Best.

At first, we observed the sugar metabolism of the normal human leucocyte culture as we find the carbohydrates in the normal human plasm. Without addition of any substances, certain glycogen deposits are already visible in the cultivated cells, just as they are in the normal leucocytes of the circulating blood. But in our researches, we have always convinced ourselves that the leucocytes did not show any glycogen granulations before the cultivation. As to the glycogen deposition in the normal leucocyte culture, we have found that, on the first two days of cultivation, a real glycogen storage is visible in the emigrating leucocytes. Both the macrophages and the granulocytes are now full of glycogen. But on the third day, the glycogen synthesis has been completed. In the next days, the cells show themselves free of glycogen. It may be concluded that, from that period, the blood sugar of the medium is consumed.

Sugar assimilation by the cultivated cells is also visible after addition of the different carbohydrates. Under these circumstances, the glycogen storage may be higher or lower in comparison with the glycogen metabolism in the normal leucocyte culture. The administration of carbohydrate may stop cell emigration and diminish glycogen storage.

The addition of glucose scarcely causes any glycogen deposition, there being only very scant amounts in the macrophages on the fifth day. Lactose does not effect a storage of glycogen at any time. But after addition of dextrose the glycogen deposition is more distinct. It makes its appearance especially in the macrophages but, in the last days, these cells also are free from glycogen. After fructose, we observe a complete absence of glycogen assimilation, also after addition of galactose and dextrin, while the glycogen synthesis is very insignificant after starch. Even the administration of alcohol does not effect a strong glycogen deposition in the human leucocyte culture. The glycogen is found in smaller quantities than in the normal culture without any additions to the medium. The glycogen deposition after maltose is only visible on the third day of cultivation.

On the other hand, we have found two kinds of sugar which effect very strong glycogen deposition in the cultivated cells. After administration of glycogen itself, we can establish a real and strong glycogen storage. Glycerin also causes a strong storage. We think that this is not a simple storage of the added glycogen itself. On the fourth day, we find glycogen as fine granules within the cells while larger granules are to be seen on the fifth day of cultivation. After administration of glycerin, glycogen is visible very strongly until the fifth day of cultivation.

Consequently, it develops from our researches that glycogen synthesis from the different sugars is brought about only under certain circumstances, that only

a few sugars are capable of building up glycogen. Many other sugars do not cause glycogen synthesis, or build up only very small quantities of glycogen. We cannot conclude that after these latter sugars, the sugar consumption is reduced, for there is the possibility that the sugar metabolism ensues in another way.

There are different hormones and irritating substances strongly influencing the sugar metabolism of the living organism, so that an augmentation or a diminution of the sugar metabolism takes place. We have examined insulin and thyroxin as to their influence on sugar metabolism in the human leucocyte culture, also adrenalin and tonephin. Helpine represents a lecithin emulsion which has an insulin-like action upon sugar metabolism (Magath). Finally, we have examined yeast which substance possesses a similar action and therefore is employed for the treatment of diabetes.

At first we examined the influence of these irritating substances without addition of any sugars to the medium. These substances did not cause any glycogen synthesis in the cultivated cells. The glycogen storage was less pronounced than under completely normal influences. Only under insulin and tonephin could we find a real glycogen storage on the second day. But on the following day, these storages had completely disappeared.

When to the human leucocyte cultures, we added sugars together with these hormones, the reactions were of another character. We have already mentioned that the administration of glucose diminishes the glycogen storage in the leucocyte culture. Now, under the influence of insulin, very distinct storage of glycogen becomes evident. Helpine caused a more pronounced glycogen storage within these cells. Especially the macrophages show these metabolic products; but storage persists only until the third day. The other stimulating substances do not cause any glycogen storage in the cultivated leucocytes after addition of glucose itself.

After the isolated administration of lactose, no glycogen storage is evident. After addition of the different stimulating substances, no change in the microscopic sugar metabolism was observed. Only heated insulin and yeast extract cause the production of very fine glycogen granules for a few days. Dextrose has effected very distinct glycogen deposition after its addition to the medium. But under simultaneous action of insulin, glycogen storage becomes very strong. In the first day, glycogen deposition appears especially in the macrophages, where the nucleus may be completely covered by that substance. On the third day, deposition is evident only as fine granulation. Afterward, no more glycogen deposition is evident. A very remarkable fact in these researches is the augmentation of glycogen deposition in the cultivated cells after helpine. Our other stimulating substances have not caused a complete disappearance of glycogen storage. Fructose does not effect any storage. Under the influence of insulin, a very strong glycogen deposition is evident, especially on the third day. Sometimes a deposit in the macrophages is visible after addition of helpine, but not in such a degree as after insulin. After thyroxin, a very distinct glycogen deposition is evident in the macrophages only on the first day of cultiva-

tion. Maltose effected glycogen storage only on the third day. Under the action of most of our stimulating substances, these depositions could not be observed at all. Only under the influence of insulin and of adrenalin small depositions are evident in the macrophages.

Glycogen deposition under the influence of the glycogen itself is very strong as already mentioned. After addition of the other substances, we can observe a distinct diminution of glycogen deposition in nearly all cases. That fact is especially pronounced after addition of insulin and of helpine although these substances usually increase glycogen storages in the cells. Under the influence of glycogen, the strongest glycogen deposits were caused by simultaneous administration of thyroxin. Throughout nearly the whole time of cultivation, a very fine glycogen granulation was evident in the macrophages and in the granulocytes. After yeast and after adrenalin, no glycogen deposits were evident at all.

After addition of glycerin, deposition also was very strong, especially in the first five days. Under addition of insulin, the glycogen deposition is of the same character in the first days, the emigrating leucocytes showing a very heavy glycogen granulation. After helpine, storage is very unimportant and only visible for some short periods. The same results have been observed after thyroxin. Glycogen storage after yeast extract and after tonephin is only insignificant. Adrenalin does not effect any glycogen synthesis in these cultivated cells. After addition of alcohol, storage was very small in the cells. Only in the first days, glycogen granules were visible. After insulin, we did not find any glycogen storage in these cells during the whole time of cultivation. Adrenalin and tonephin did not effect glycogen storage in these cells. But very distinct glycogen granules were evident after helpine. Storages were more extensive than after insulin. The macrophages were as full of glycogen granules as the granulocytes in the first days. But afterward a complete disappearance of the glycogen deposition took place.

After simultaneous administration of insulin and galactose, glycogen storage increased strongly in all the periods. But from the sixth day, a complete disappearance was evident. Synthesis after helpine is very insignificant; it is a little stronger after thyroxin where especially the polynuclear leucocytes show very distinct glycogen depositions on the first and on the fifth days. Storage is insignificant after tonephin, adrenalin, and yeast extract.

Dextrine administration was not accompanied by glycogen deposition in the human leucocyte culture. Under the simultaneous influence of our hormones, glycogen metabolism did not change. Only under the influence of insulin could we observe some glycogen granulations in the granulocytes on the first day, while the other cells of the same culture were free; but in the following days we could not find any glycogen.

After administration of starch we could normally observe distinct glycogen granules within the macrophages on the third day. Under the simultaneous influence of our hormones we could not see such a reaction. Glycogen deposition was of a very strong character after insulin, especially in the macrophages. Sometimes storage was of so great an intensity as to cover all the other cell structures. After helpine complete absence of glycogen was evident. The same

microscopic findings were present for yeast, tonephin, and adrenalin, but thyroxin effected a real glycogen storage in the macrophages and in the granulocytes during the whole cultivation time.

So we can recognize in these researches that there is no uniform action of the stimulating substances upon the metabolism of the different sugars. After insulin, we observed an augmentation of the glycogen storage only after addition of sugars such as glucose, dextrose, fructose, galactose, while, after addition of other sugars, an augmentated glycogen synthesis is never observed. As to the action of insulin, this is not necessarily contradictory to its effect in the living organism, because we have applied only small doses of insulin under these circumstances. On the other hand, morphologic insulin metabolism has been observed only in the liver cells of the whole organism. It is said that helpine shows an insulin-like action. In our researches on the human leucocyte culture we established glycogen synthesis with helpine, sometimes more pronounced than that caused by insulin. This is especially noticeable after administration of glucose and of alcohol. As to the other sugars, glycogen synthesis may be suppressed completely by helpine. Yeast is said to also show an insulin-like action, diminishing the blood sugar in the living organism. In our researches, we observed that glycogen storage in the human leucocyte culture did not increase after yeast. Only in our series with lactose, a transitory glycogen storage took place within the granulocytes.

There is a diminution of glycogen deposition in the liver cells after administration of thyroxin to the living organism. In the cultures there was an increase in glycogen after simultaneous administration of glucose although this synthesis appeared in the last periods of cultivation. An unimportant storage took place after thyroxin and the sugars: glycerin, fructose, galactose, and alcohol. Sometimes glycogen synthesis under thyroxin surpassed the storage after insulin. Glycogen storage is especially strong after addition of glycogen itself to thyroxin. The same results were to be found after starch.

We must answer the question if the absence of glycogen in the cultivated cells is to be regarded as a sign of an absent carbohydrate synthesis. We may think that a lack of glycogen reveals a very strong digestion of the carbohydrates, but other metabolic products may develop which are not visible by microscopic methods. Certainly, a negative finding as to the glycogen deposition can only be regarded as a negative finding for itself.

Nevertheless, we feel that we have pointed out a new way for the study of carbohydrate metabolism. From these researches, it results that the microscopic method reveals a great many new facts regarding the metabolism of the cells. Therefore, the microscopic method should be employed much more in the study of the metabolism of the organism.

CONCLUSIONS

The sugar metabolism of the human leucocyte culture may be studied by the morphologic observation of glycogen storage in the cultivated cells.

We have demonstrated that glycogen synthesis from the different sugars varies greatly. We observed strongest glycogen storage after administration of

glycerin and of glycogen itself. Insulin can be regarded as a glycogenetic substance in the human leucocyte culture only after simultaneous addition of certain sugars. Otherwise a diminution or no change of the glycogen deposit took place. Another substance of a certain glycogenetic activity was a lecithin emulsion, called helpine.

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NEUFELD REACTION IN CERTAIN CASES OF PNEUMOCOCCIC SEPTICEMIA*

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THE blood culture in some cases of pneumonia appears to offer the only medium of rapid determination of type. Random cases of infection which have entered this hospital for observation during the past two years have proved to be respiratory pneumococcic infection of atypical clinical course which could not be typed in the usual manner by means of sputum because in some expectoration was absent. Blood cultures, however, were positive for organisms of the streptococcal-pneumococcal groups within eighteen hours.

Diffuse growth of the organisms occurred in initial broth culture of the blood, yet attempts to determine type by means of bile solubility, macroscopic agglutination, or precipitin tests failed. Exhibition of failure of the tests was evidenced when the tubes were placed in water-bath and opaque yellowing precipitates settled out in a few minutes. These precipitates of nonspecific unknown identity interfered, and this interference arose apparently from the patient's blood stream constituents. They suggested heavy colloidal suspensions being thrown out of the dispersed state. The difficulty was eliminated by preparation of initial saline suspensions from centrifugalized sediment of the initial supernatant blood broth culture. Bile solubility and Neufeld tests were made from the saline suspensions. Neufeld tests were satisfactory in each instance, 100 per cent of the pneumococci showing encapsulation in specific serums. Mouse inoculation tests of such cultures were unnecessary, as well as preparation of subcultures for purpose of elimination of the interfering substances.

The precipitin test of Krumwiede and Valentine was performed upon sputums and blood culture cell sediment from many of the cases. For some

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unexplained reason, there have been few positive precipitin tests for the past two winter seasons. This finding has been concurrent with atypical clinical pneumonias.

Preparation of saline suspension of all streptococcal-pneumococcal organisms isolated from blood were made for determination of type of pneumococci by means of bile solubility and Neufeld tests. The precipitin test of Krumwiede and Valentine was set up as a comparative procedure. Immune rabbit serum for the tests were obtained from Miss G. Cooper of the Research Laboratories of the Department of Health, New York City, and from Lederle Laboratories of New York.

THE DOCTOR AS CONTRIBUTOR TO CIVILIZATION*

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THE doctor's contribution to the welfare of humanity in extramedical spheres are many and of the greatest value and interest. As explorer, as philosopher, as sociologist, as musician he has contributed to the advance of progress. In this article we shall review some of these many-sided activities of the doctor.

THE DOCTOR AS EXPLORER

The earliest of great doctor-explorers was Mungo Park, born in 1771. At fifteen years of age he was apprenticed to a surgeon, Thomas Anderson by name, under whom he studied for several years. He later continued his medical studies at Edinburgh where he obtained his medical degree. In 1791 he went to London where he obtained an appointment as assistant medical officer on the "Worcester." In 1792 he sailed to the East Indies, returning one year later with rare plants and animals from Sumatra which he presented to his patron, Sir Joseph Banks.

By 1795 the reputation of Mungo Park as an explorer was fully established. In that year he was sent by the African Association to find the course and termination of the Niger River. He had many adventures on that voyage of exploration. In July of that year he arrived at Pisiana, 200 miles up the Gambia. Six months later he started on a journey of adventure and discovery, accompanied by a negro servant and a boy. He was robbed by the natives through whose territory he passed. He was lucky to get away alive. For four months he was kept a prisoner by an Arab chief, from whom he ultimately escaped.

Mungo Park was the first extensive explorer in the mysterious continent of Africa. He was the first European of modern times to strike the Niger and his work, more than any other's, did most to open up the dark continent to exploration and settlement by the white man.

The most famous of doctor explorers was David Livingstone (1813-1873) who began life in a cotton factory at the age of ten. His interest in medical

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missionary work asserted itself early. He enlisted with the London Missionary Society and studied medicine in London, becoming a licentiate of the Faculty of Physicians and Surgeons of Glasgow in 1840.

Immediately upon graduation Livingstone sailed on the first of his three great trips of exploration to Africa. It was his great ambition to open up Africa to commerce and to abolish its slave trade evil. He wanted not only to show the advantages of Africa to the white man but also to better the conditions of the native black man.

Dr. Livingstone died at Ilala on Lake Bangweulu in 1873. His heart was removed from his body by his faithful native followers and buried under a tree. His embalmed and dried body was then brought after a long and hard journey to the coast whence it was sent to England to rest forever in Westminster Abbey.

Another notable doctor explorer was Sir John Richardson (1787-1865) who at the early age of twelve was apprenticed to his uncle who was a surgeon. Later he studied and qualified as a member of the Royal College of Surgeons in London. In medical practice he was not very successful and for that reason he applied for appointment as surgeon and naturalist to the first Arctic expedition of Sir John Franklin. He was second in command. From 1825 to 1827 Dr. Richardson was with Franklin on his second voyage of exploration. He was the first scientist to make a thorough study of Arctic flora and fauna.

THE DOCTOR AS PHILOSOPHER

To philosophy and psychology the doctor has been a significant contributor. John Locke (1632-1704) was one of the greatest of early English philosophers. He received his M.A. from Oxford in 1658, and he became a lecturer at that university in Greek and rhetoric. Later he decided to study medicine. In 1668 he became an F.R.S. and in 1674 he received his M.B. and practiced medicine for several years. He was an intimate friend of the great English clinician Thomas Sydenham and often accompanied him in his rounds. He wrote a Latin poem for the second edition of Sydenham's work on fevers.

Dr. Locke's greatest work is entitled *An Essay Concerning Humane Understanding,* which was published in 1690. Twenty editions of this remarkable work appeared by the end of the eighteenth century. It was translated into French, Latin, and German. Locke was recognized as the leading philosopher of his time. Another important book, *Epistola de Tolerantia,* was published in Latin. It was soon translated into other languages. Dr. Locke was one of the really great philosophers of all time.

In Germany Rudolf Hermann Lotze (1817-1881) held a similar position. He was a graduate of the medical faculty of Leipzig, but his interests lay mainly in philosophical studies in which he established an enviable reputation for himself.

In this country the medical profession is represented by William James (1842-1910) who was one of the greatest psychologists of modern times. William James studied medicine at Harvard from which he received his M.D., but he never practiced his profession. He at first taught anatomy and physiology at his Alma Mater, but later his interests were attracted to philosophy and psychol-

ogy, which subjects he also taught at Harvard. He became the Professor of Psychology in 1885. He is the author of an imposing list of very important books: *Principles of Psychology, The Varieties of Religious Experience, Pragmatism.*

THE DOCTOR AS MUSICIAN

Among the earliest of noted English composers was George Ethridge who lived during the sixteenth century and who was one of the most famous vocal and instrumental musicians of his day. He was a graduate of Oxford and a physician of no little ability.

Among the earliest compositions extant by medical men are three songs by Thomas Campion. These are dated 1596 and are in the Harleian Manuscripts 6910 (British Museum). Campion took the degree of Doctor of Medicine, apparently at Cambridge, and had previously studied for the legal profession. He also published four books of "Ayres" which have given him a place in musical biography. He was also a musical theorist of note; his *New Way of Making Foure Parts in Counterpart, by a Most Familiar and Infallible Rule,* published about 1618, went through many editions.

Twenty years before Campion's death the will of Sir Thomas Gresham, establishing a Professorship of Music, had taken effect. The first five men to hold this chair were all physicians.

Probably the medical man whose name is most familiar to music lovers is Thomas Harington. His *Great Is the Pleasure* is one of the most popular of musical compositions, and it has been played and sung in all quarters of the globe. He was born in 1727 at Kelston, Somerset, England. He had at first intended to study for the Holy Orders but later changed his mind and qualified as M.D. at Oxford. During his college years he became very much interested in music.

Dr. Harington was most successful in all his undertakings. His life was spent chiefly in Bath, where he was a prominent physician. He also became much interested in civic affairs and was first alderman and later mayor of his city. All his leisure was devoted to music and composition. He composed much music during his lifetime, most of which became very popular.

Another early English doctor-composer was William Kitchener who was born in 1775 and who took his M.D. at Glasgow University. Dr. Kitchener composed not only songs but also more ambitious works. He composed a musical drama under the name *Ivanhoe* and an operetta *Love Among the Roses.* He died in 1827.

By no means were the doctor-musicians limited to England. Florent Corneille Kist, born at Arnheim, Holland, in 1796, studied medicine and practiced as a doctor at The Hague until 1825, after which he devoted himself entirely to music. His compositions consist of vocal pieces for one and several voices, and a volume of variations for the flute, on which he was an excellent performer. But it is chiefly as an organizer of musical societies at Delft, The Hague, and Utrecht and founder of the *Caecilia,* which is still the most important musical paper in Holland, that he is known.

OTHER ACTIVITIES

The extramedical activities of physicians have been most varied and significant. There is scarcely a field of human endeavor which has not felt the touch of the doctor's hand. Fire insurance was originated by a medical man. Nicholas Barbon was born in London and studied medicine at Leyden. In 1661 he graduated (M.D.) at Utrecht and three years later he was admitted to the fellowship of the College of Physicians. For a time he was a member of Parliament. After the Great Fire of 1666 he was one of the principal builders of the city of London. What he is most remembered for was that he was the first projector of fire insurance.

One of the great pioneers of South Africa was William Guybon Atherstone (1814-1898). It was from his researches that the great diamond mining activity in South Africa was initiated. In 1867 he identified the first diamond found at Colesburg-Kop, now known as Kimberley. Dr. Atherstone examined the diamond with a polariscope and tested its hardness on a windowpane. The diamond was later sold for $4,000. The original windowpane upon which the diamond was tested has been framed and is still preserved as a memento of that great occasion. In 1888 the Kimberley companies presented the pioneering physician with a valuable diamond as a token of appreciation for his work in founding a great industry.

To chemistry, doctors have made some very important contributions. As space is limited only three will be referred to. Thomas Andrews was the first to demonstrate the true nature of ozone, proving it to be an allotropic form of oxygen. Joseph Black is immortalized as the discoverer of latent heat in 1761. He also originated the theory of specific heat. In 1767 Dr. Black made the first attempt to inflate a balloon with hydrogen.

Another notable chemist was Daniel Rutherford (1749-1819) who was born in Edinburgh and received his medical education from the university in that city. His thesis for the M.D. degree in 1772 was significant because of the clear distinction he made between carbonic acid gas and nitrogen. He was also the first to isolate the gas nitrogen by burning substances in an enclosed volume of air and absorbing the carbonic acid thus formed.

By no means are the extramedical activities of doctors covered in this and the preceding articles. Nor have all the names been mentioned. To do this would have been a most ambitious undertaking and a most comprehensive work. These articles have served merely to call attention to the fact that doctors have made great contributions not only to medicine but to almost every field of human endeavor. The extramedical contributions of the medical profession have been almost as important, perhaps just as important, as their purely medical contributions.

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HEMATOLOGIC NOMENCLATURE*

"Teknocyte" and "Korocyte" Suggested in Emendation of the Designations "Juvenile" and "Stab" in the Schilling Hemogram

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THE validity and practical worth of Schilling's[1, 2] contribution to the clinical study of infections and intoxications are being constantly more widely recognized. Simplicity is often mentioned as one of the points in its favor, just as complexity may have caused neglect of its predecessor, the Arneth[3] count. Much more important, however, are differences of interpretation based upon the regenerative and degenerative influences revealed by the appearance and number of the ontogenetic types of neutrophiles enumerated. Arneth grouped his three young cells (M, W, T) and separated the various mature neutrophiles in accordance with their nuclear structure. Schilling, on the other hand, separated Arneth's three young types, with minor differences in cleavage, and grouped the polymorphonuclear neutrophiles; thus he had the basis for an analysis of the influences which alter not only the development but also the production of the granulocytes.

The several attempts that have been made to reduce the time consumed in making complete Schilling hemograms have not been quite successful, because valuable information has been sacrificed to save a negligible amount of time. All cells present must be counted, and any abnormality of appearance noted; whether the tally is put down in one column more or less can make little difference. In any laboratory, the blood specimens in which there is a marked "shift to the left" will usually be exceptional; when such do occur the information gained by careful study is worth more to the clinician than it costs in extra time. This point (of the relative negligibility of the time factor) receives emphasis from the work of those who are restudying intensively the cytoplasmic changes in the granulocytes.[4-8] That any consolidation of the three young forms of Arneth (M, W, T) or of Schilling (myelocyte, juvenile, stab) may reduce the value of the count disproportionately is made clear by Piney.[9] If further evidence is needed to support this statement it is furnished amply by study of the figures in the first three columns under Class I, of Arneth's original protocols (although only the granulocytes are counted) with their respective case histories. Schilling's own work is still more convincing, of course. Appreciative notation might also be made here of the recent paper by Crocker and Valentine.[10]

However, there is a point in connection with the Schilling hemogram which demands emendation. This is the matter of nomenclature. The language of hematology has otherwise remained remarkably free from the taint

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of medical slang. In the past, writers have not hesitated to apply accurately descriptive terms, of Greek derivation, to the blood cells as shown by the current use of such words as "poikilocytosis" and "anisocytosis."

Even though the words "juvenile" (jugendlich) and "stab" have been in use since 1912,[1] when the first edition of Schilling's work was published in Germany, and especially since 1929 when Gradwohl's[2] translation of the seventh and eighth revised edition was published in this country, they cannot be considered as anything but a part of the convenient and careless argot of the laboratory. Furthermore, they have by no means found general acceptance.

Whether or not there is universal agreement as to the technic, significance and limitations of the Schilling differential count, or whatever modification of it the future may bring about, the morphologic types called by Schilling "juvenile" and "stab" represent significant developmental stages in maturation between the myelocyte and the polymorphonuclear leucocyte. As such they deserve designations in conformity with the nomenclature of biology, designations, which, strange to say, they have never received.

In seeking suitable names we have no fixed precedent as to the important characteristic of a blood corpuscle which shall be represented. The words myeloblast and myelocyte refer to the place of origin of these cells, with "blast" and "cyte" designating their respective ages or state of maturation. "Polymorphonuclear" describes the most striking and characteristic appearance of this mature leucocyte, while "neutrophilic" (heterophilic),[11] "eosinophilic," and "basophilic" refer to chemical reactions of cytoplasmic granules in the respective formed elements of the blood.

So far as their significance in clinical pathology is concerned the important characteristic of the "juvenile" and "stab" cells is their developmental age. The younger of the two, the "juvenile" of Schilling, in its age relationships is like the Greek word τέκνον: that which is born, a child; the somewhat older cell, but still immature in the form and shape of its nucleus, the "stab nuclear" of Schilling, seems to correspond in relative age with the Greek κόρος: a boy, a lad, up to the military age.

The adoption of the following into the nomenclature of hematology is therefore recommended:

Teknocyte (τέκνον + κύτος), the young granulocyte, rarely found in the circulating blood in health (0 – 1 per cent), which follows ontogenetically immediately after the myelocyte. It is somewhat larger than the mature cell; its protoplasm is like that of the older granulocyte; the nucleus varies in form from sausage shape nearly to bean shape. The chromatin does not stain quite so deeply as that of the mature cell but it is distinctly subdivided into fields, with definite nuclear bodies in the end bulbs (circumscribed chromatic nucleoli). It is identical with the Schilling "juvenile neutrophilic leucocyte."

Korocyte (κόρος + κύτος), the youngest granulocyte found regularly in the circulating blood in health (3 – 5 per cent). In ontogenetic development it is between the teknocyte and the polymorphonuclear. In size it is barely larger than the mature granulocyte and the cytoplasm of both is alike in

appearance. The nucleus is narrower, more shriveled, than that of the teknocyte and in shape may resemble the letters and figures C, U, V, S, Z, W, T, E, 6, 8, 3. Segmentation is never so nearly complete as in the polymorphonuclears, the lobes are connected by bands broader than a thread. The margins are uneven and the chromatin is gathered in irregular, dark compact masses between which light apertures appear. There are no nucleoli. It is identical with the Schilling "stab nuclear leucocyte."

The degenerative korocyte (degenerative Stabkernige) is a neutrophile which due to degenerative inhibition of development has matured without segmentation. It is distinguished from the normal korocyte by cytoplasmic differences and by its narrow hyperchromatic and pyknotic nucleus which is sometimes curved into bizarre shapes. This cell is considered of special significance in the hemogram. Recognition of degenerative changes in it does not, however, preclude observation of such changes in the other cells as have been so carefully recorded by Naegeli[19] and Gloor.[20*]

In common with the other granulocytes, teknocytes and korocytes may be neutrophilic, eosinophilic or basophilic.

These young cells were among those described and discussed by Ehrlich.[12] They were known as transitional forms of the "ε" granulocytes but no one had attempted an orderly arrangement of them prior to the work of Arneth.[3] His great contribution to hematology was the result of careful morphologic studies which culminated in the "Arneth count." This, as is well known, correlates the differential count of the various nuclear forms, in the circulating blood, with disease. The proportions of old and young forms were so related to infectious conditions that the count was of value not only in diagnosis but in prognosis as well. He used the term "shift to the left" to denote the increase in young forms which he found to occur regularly in severe pyogenic infections and certain other conditions. He separated the neutrophiles into five classes according to the configuration of their nuclei. The three youngest forms he grouped together as Class I, designating the myelocyte as "M," the younger of the other two cells as "W" (Zellen mit wenig eingebuchtetem Kerne), the older as "T" (Zellen mit tief eingebuchtetem Kerne). Pappenheim[13] had concerned himself more with the ontogenesis of the blood cells than with their relationship to clinical pathology. To him the group of forms included under Arneth's "W" and "T" types were merely cells which follow the myelocytes in development toward maturity. Therefore, all the intermediate forms he called "Arneth cells" or "metamyelocytes," (*μέτα*—after). He also called the cells immediately preceding the myelocytes in age "promyelocytes," (*πρό*—before).

Pappenheim's interest was purely scientific; Schilling's, like Arneth's, was practical, the application of blood counts to disease. Therefore Schilling agreed with Arneth in recognizing two intermediate types or two age forms instead of one. Schilling at first[1, 14] considered the "juvenile" forms identical with metamyelocytes but later[2] stated that "They are nearly identi-

*The description of the teknocyte follows closely that given by Schilling,[2] while details of the korocyte are taken largely from Crocker and Valentine.[10]

cal with Pappenheim's metamyelocytes, which, however, are not clearly outlined by the author." Study of various forms in Pappenheim's Atlas,[13] inclines one to agree with this statement, since the cells shown as metamyelocytes cover the entire range between myelocyte and polymorphonuclear. With regard to his "stab nuclear" Schilling does not agree that it is identical with Arneth's T; he seems to consider the younger of W's to be true myelocytes and the older to be stabs. The lack of agreement between Arneth and Schilling upon these points is probably no greater than between any other two hematologists concerning the Schilling types. There can be no sharp, natural, differentiating criteria because the forms in question are developmental stages. There is need for cooperative work by a committee of interested persons to establish the characteristics of these two important types and eliminate as far as possible local differences among the accepted pattern cells of the various laboratories.

Uncertainty as to differentiation has undoubtedly had much to do with suggestions that the teknocytes and korocytes be counted together as metamyelocytes, or by some other name. Furthermore, nearly every worker, unable to see a band or a rod in the stab (!) shaped nucleus, has invented his own designations. None of the names suggested has been constructed in accordance with the traditions of biologic nomenclature; a few of them are tabulated below. The upper part of the table is an adaptation of one arranged by Schilling.[14]

TABLE I

	MYELOCYTES	TEKNOCYTES	KOROCYTES	POLYMORPHONUCLEARS
			DEGENERATIVE KOROCYTES	
Schilling[1, 14]	Myelozyten	Jugendliche	Stabkernige	Segment kernige
			Degenerative Stabkernige	
Gradwohl[2]	Myelocytes	Juveniles	Rod or Staff nuclears	Segment nuclears
			Degenerative stab or staff forms	
Arneth;[3] Schilling[14]	M	W	T	Segmented Classes II-V
Pappenheim[13]	Myelozyten	Metamyelozyten		Polymorphonuclearen
Cooke and Ponder[15]	–	–	Class I	Class II-V
Piney[9]	Myelocytes	Young forms of metamyelocytes	Band forms of metamyelocytes	Polymorphs
Pons and Krumbhaar[16]	Metamyelocytes (very young)		Nonsegmented forms (young)	Segmented forms (older)
Farley, St. Clair, and Reisinger[17]	Myelocytes	Nonfilament nuclear types		Filament nuclear types
Kolmer and Boerner[18]	Myelocytes ?	Y or young types	B or band types	Neutrophiles
Crocker and Valentine[10]	Myelocytes	Juveniles	Stabs	Segmenters

In the volume by Cooke and Ponder,[15] as Fig. 2, is "a graphic representation of Arneth's scheme taken from Gruner's *Biology of the Blood Cells*." This drawing is interesting because it shows that, as interpreted by some

workers, there was no correlation between the Arneth types and those of Schilling. Cells considered as Class III of Arneth would be called stabs by Schilling. Cooke's modification of the Arneth count is also illustrated in this volume. No cell younger than a korocyte is shown.

One of the methods suggested to simplify the Arneth count is that of Pons and Krumbhaar.[16] They divide the neutrophiles into: "(1) metamyelocytes (very young), with round or slightly indented nuclei; (2) nonsegmented forms (young), where the nuclear material is connected by broad bands; (3) segmented forms (older), where two or more groups of nuclear material are joined by narrow filaments." The illustration shows, as Group 1, forms which would generally be called myelocytes; in Group 2, the forms pictured are similar to those shown by Pappenheim[13] and called by him "metamyelocytes"; some of them would be called by Schilling "juvenile" and the others "stabs." The reason for suggesting such a "shift" in terminology is not clear.

More recently, Farley, St. Clair, and Reisinger[17] have suggested a simplification of previous methods by dividing the polymorphonuclear neutrophiles into "filament" and "nonfilament" nuclear types. This is said to be based more directly upon the work of Cooke and Ponder.[15] They reprint the illustration from Cooke and Ponder which shows Class 1 to be composed of korocytes. The text states, however, "Distinct myelocytes or myeloblasts are to be counted as such."

In the volume, *Approved Laboratory Technic*, prepared under the auspices of the American Society of Clinical Pathologists, Kolmer and Boerner[18] state, on p. 82, "Schilling has recommended a division of the metamyelocytes into two types, namely, (a) young forms with round or slightly indented nucleus corresponding to Arneth's M and W cells, and (b) band forms with deeply indented or bandlike nuclei which correspond to Arneth's T cells (Fig. 60)." Actually, Arneth[3] used the "M" as the initial letter of the word "myelocyte" —"M soll Myelocyten - - - bedeuten" (p. 17). These authors (p. 82) suggest "Y" and "B" as designations for the young granulocytes: "Namely, (a) those with a slightly indented nucleus called young or Y types and (b) those with a deeply indented nucleus called band or B type." It is not quite certain, for Fig. 66 offers no help, but it seems that this classification resembles Schilling's and not that of Pons and Krumbhaar[16] as the statement concerning Schilling's division of the metamyelocytes prepares one to expect.

This brief review is believed sufficient to indicate the carelessness that has crept into hematologic nomenclature and literature and to emphasize adequately the need for a return to classic terminology and an orderly scientific language.

SUMMARY

1. The methods suggested as substitutes for the Schilling hemogram save an insignificant amount of time at the expense of valuable information.

2. The immature granulocytes called by Schilling "juvenile" and "stab nuclear" have never received designations in conformity with scientific hematologic nomenclature.

3. It is recommended that the word "teknocyte" be substituted for "juvenile" and "korocyte" for "stab nuclear."

4. There is need for greater care in the construction and use of the technical terms of hematology.

The kind interest and assistance of the Reverend J. Coleman Nevils, S. J., President of Georgetown University, in the search for suitable root words is gratefully acknowledged.

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LABORATORY METHODS

A NEW METHOD FOR THE DETERMINATION OF CUTANEOUS CAPILLARY BLOOD PRESSURE*

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MANY methods are available for the determination of the blood pressure in capillaries. The most direct method is probably that of Carrier and Rehberg[1] in which a minute tube filled with saline connected to a manometer is inserted directly into a capillary, and the entrance of blood or exit of salt solution at different pressures is noted. This method, while direct, is only suitable for research purposes. The method of Danzer and Hooker[2] is also excellent but requires rather elaborate equipment (Duryee and Wright[3]). These authors observe through a transparent pressure capsule the movements of corpuscles in individual capillaries. The pressure found necessary to stop

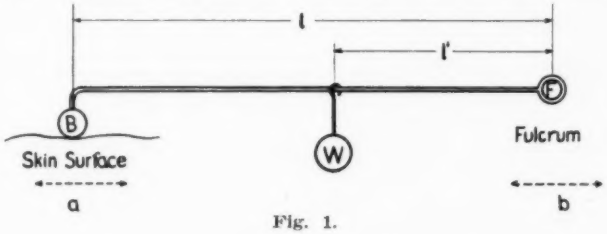

Fig. 1.

the flow of corpuscles in the capillaries is found to be very much greater than the pressure that produces paling of the skin. Paling of the skin is used in the method of von Kries[4] which consists essentially of weighting a glass of known area until the capillaries' bed under the glass is bleached. This method is subject to the criticism that during the loading period the capillary bed is more or less occluded and subject to stasis of the blood, and in addition, the area of glass applied to the skin is variable, due to the topography of the skin. Other methods are based essentially on this same method and are subject to error due to the resistance of the cutaneous tissues to deformation.

The new method about to be described has yielded results comparable to those of Carrier and Rehberg. Such results have been obtained by students as well as by trained investigators. The instrument (see Fig. 1) consists of an arm hinged at one end to an upright and bearing on the other end a small glass bead. The arm is bent downward at right angles to carry the bead. In operation the glass bead (B) is applied to the skin by a sliding weight (W) on the calibrated arm (l). The skin surface is moved, as indicated by arrows a,

*From the Physiology Department of Stanford University.
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at a moderate speed with the glass ball riding over the skin. When the skin surface itself cannot be easily moved, the ball may be made to make excursions back and forth (b) by having the fulcrum (f) of the lever on a ratchet and pinion. If a white flash appears, as the ball passes by, the weight is moved nearer the fulcrum until the pink color of the skin is undisturbed by the weights. These white flashes are of very short duration and are not to be confused with the local vasomotor reaction to strong mechanical stimuli, which has a latent period and duration of many seconds (Krogh[5]).

The actual surface of the ball that makes contact is determined by noting the diameter of the ink spot on the ball after application to the ink-smeared skin. This area divided into the pressure, calculated by the law of levers, gives a value convertible into mm. Hg.

$$\frac{Wl'}{l} + \frac{\text{wt. lever}}{2} = P$$

$$\frac{P \text{ in milligrams}}{\text{area in sq. mm.} \times 0.0136} = \text{mm. Hg}$$

The method avoids the danger of stasis, gives a statistical average surface that is constant as the ball passes rapidly over cutaneous areas of different contour and variable deformability. The adjustment of pressure is convenient and accurate.

The importance of determination of capillary blood pressure in clinical work may be expected to increase. Already this measurement is of importance in urticaria, inflammation, circulatory shock, edema, etc. (Krogh[5]).

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THE LIMITATIONS OF COLORIMETRIC ANALYSES BY PRESENT METHODS*

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IN DOING routine colorimetric determinations, the authors have been impressed by the frequent discrepancies in readings obtained with slight differences in technic. The following data are an attempt to create a more general appreciation of the inherent limitations to the accuracy of various routine colorimetric analyses. These limitations are the result of many chemical factors, the most important of which are correlated in Table I. The data presented in Table I have been selected from many hundreds of analyses made over a period of a year. The test results selected from this large group were chosen because they best illustrate the factors of error involved, and each represents an average reading resulting with the correlated factor. The figures and notes have been arranged, purposely not in chronologic order, but rather in a sequence which tends to show the cumulative effect of these prominent factors.

We might digress to explain that colorimetric chemistry is that science which deals with the quantitative analysis of unknown chemicals in terms of color reactions. These color reactions are derived by a special treatment of the substance under analysis with a reagent which is often very complicated and which produces a color reaction whose intensity varies in relation to the quantity of the substance being analyzed. This method of quantitative chemical analysis, although in general a relatively simple procedure for the clinical technician, nevertheless involves many technical factors (often beyond the control of the operator) which influence the degree of precision of the readings. These types of analyses even if handled with the extreme care and caution of a skilled analytical chemist, are not free from some erratic results. The following exemplary data taken from an experimental notebook will tend to show some of the factors normally overlooked by the routine clinical technician.

The data were obtained through the use of a new type of photo-electric colorimeter of the author's design, the advantages of which will be described below. With this instrument it was possible to obtain color density readings without intercomparison with a standard, thus making it possible to measure accurately the influence of such factors as treating technic, temperature, time, concentration of reagents, etc. It can be indisputably proved (see description of colorimeter) as far as these data are concerned that the precision of the color density readings of this instrument was of a much higher order of accuracy than the errors or percentage differences which are shown in the table. These errors, therefore, are essentially the result of some erratic chemistry, which the authors

*From the Department of Pathology, Hamot Hospital and the U. S. Bureau of Standards. Received for publication, December 8, 1934.

have attempted to correlate with the major causative factors. It should be obvious from this table that the reaction technic alone which involves the factors under columns five and six may routinely introduce errors of at least 5 to 10 per cent.

TABLE I

ACTUAL WT. OF SPECIMEN	NO.	EQUIVALENT COLOR DENSITY READING	% OF ERROR	SIZE OF CONSTRICTED NECK OF FOLIN TUBE	AGITATION AFTER REDUCTION OF CU
	BLOOD SUGAR				
200.0 mg.	A	190.0	– 5.0%	Average	Normal routine care
200.0 mg.	B	190.0	– 5.0%	Average	Normal routine care
150.0 mg.	C	140.0	– 6.6%	Average	Normal routine care
125.0 mg.	D	122.0	– 2.4%	Average	Normal routine care
100.0 mg.	E	91.0	– 9.0%	Slightly larger	Normal routine care
100.0 mg.	F	91.0	– 9.0%	Slightly larger	Normal routine care
100.0 mg.	G	89.5	–10.5%	Slightly larger	Normal routine care
100.0 mg.	H	87.5	–12.5%	Average	Slight agitation*
100.0 mg.	I	87.0	–13.0%	Average	Slight agitation*
200.0 mg.	J	166.0	–17.0%	Average	Slightly more agitation*
75.0 mg.	K	74.0	– 1.0%	Large	Normal routine care
75.0 mg.	L	75.0	0.0%	Large	Normal routine care
100.0 mg.	M	85.0	–15.0%	Very narrow	Normal routine care
50.0 mg.	N	46.0	– 8.0%	Narrow	Normal routine care
100.0 mg.	O	75.0	–25.0%	Narrow	Slight agitation*

	URIC ACID			REMARKS	TECHNIC
4.00 mg.	P	3.1	–22.5%	Reagent of Folin's modified method. (About 1 year old)	Folin's normal routine for this method
4.00 mg.	Q	3.6	–10.0%		
4.80 mg.	R	4.2	–12.0%		
6.40 mg.	S	5.3	–17.2%		
8.00 mg.	T	6.6	–17.5%		
			5)79.2		
			15.8%	is the average correction to be applied to all readings in this group	

	UREA NITROGEN			REMARKS	TECHNIC
15.00 mg.	U	14.5	– 3.3%	Fresh Nessler's reagent prepared from old mercuric iodide stock sol.	Standard routine throughout
20.00 mg.	V	18.0	–10.0%		
40.00 mg.	W	37.0	– 7.5%		
40.00 mg.	X	35.0	–12.5%		
60.00 mg.	Y	58.0	– 3.3%		
60.00 mg.	Z	57.0	– 5.0%		
80.00 mg.	AA	71.0	–11.2%		
100.00 mg.	AB	83.0	–17.0%		
120.00 mg.	AC	95.0	–21.0%		
			9)90.8		
			10.1%	is the average correction for this group	

*All cases of deliberate agitation were for a period not longer than two seconds.

The present reading technic, by means of the "Duboscq" type* of colorimeter, is supposed to eliminate such variations in readings as are shown in the table, through simultaneous treatment of a standard with the unknown. Unfortunately, however, these errors are probably only rarely eliminated by running

*The "Duboscq" type colorimeter optically compares the unknown solution with a standard, which has been similarly treated chemically.

a control simultaneously with the unknown inasmuch as certain factors are not routinely so exactly the same as it is supposed. The data presented will confirm this contention. For instance, in a blood sugar analysis, of all the tubes that are used (Standard and Control), the constricted neck in each will routinely vary considerably, thereby controlling the rate of diffusion of the phosphomolybdic acid with the initial contents (see "K," "L," "M," and "N" in the table). Also routinely one of the tubes may be shaken slightly more than the other after the reduction of the copper, causing a relatively large error for the amount of agitation, the effect of which is demonstrated by readings "H," "I," "J," and "O." The maximum agitation time was not more than two seconds in any case.

The relatively large errors which result from this agitation, makes it readily conceivable that sufficient shaking may occur routinely, to cause considerable errors in readings. In other words, if the unknown is disturbed more than the standard, the error will be negative. Whereas, if the standard is disturbed the more, the error will be positive. Or if both should receive precisely the same amount of agitation, the errors (negative and positive) due to this one factor would cancel out. This factor is so critical that the latter case rarely occurs in routine work.

When we consider the urea nitrogen analyses by Nessler, we see a colorimetric test that has been classic in being both critical and erratic, but nevertheless valuable when its shortcomings are accounted for. In these analyses there is often a distinctly different reaction involved in the unknown, relative to the standard. For instance, very small differences in the acid base equilibrium as are commonly introduced in the unknown relative to the standard will bring about a decided cloudy or imperfect color reaction in that unknown. At any rate the reaction is definitely of a different character than occurs in the standard with which it is compared. The urea nitrogen group in the table reveals the magnitude of the erroneous nature of the reaction of Nessler's reagent with even the C. P. ammonium sulphate solution that was used throughout this series.

In Folin's modified method for the analysis of uric acid, the age of the reagent is the important factor affecting the specific color density. The uric acid series in the table demonstrates the diminution in specific color density to be expected from this factor. The 15.8 per cent represents the average approximate diminution for a one-year-old reagent. (All other solutions used in conjunction with this series were fresh and standardized.) The marked deviation in the individual errors from the average (15.8 per cent) probably represents the effect of uncontrollable factors, which undoubtedly introduce erratically errors of these general magnitudes to routine tests.

It has apparently been the custom of clinical laboratories to overlook the grossness of errors introduced through these hidden factors and profess a precision of readings of the unknown of 1 per cent or better, in all these tests. Although one does not actually vouch for the accuracy of a reading, one suggests the precision of the reading by the number of significant figures which he writes in the final result. For instance, if he reports a normal blood sugar analysis to 0.1 mg., he is suggesting an overall precision within $\frac{1}{10}$ per cent. In view

of the foregoing data, figures to the right of the decimal point in readings of approximately 100 mg. or over are certainly superfluous and meaningless. In any case where a certain digit is superfluous, mathematicians have taught us to substitute a zero in its place, if it lies to the left of the decimal point, and to drop the digit entirely if it lies to the right of the point; and retain a numerical value for only those figures which are really significant. Certainly no more than three digits should ever be used to represent the results of a routine colorimetric test. The author believes that it is only jeopardizing incentive for research and ultimate improvement in colorimetric chemical methods to profess a precision within 1 per cent or better in the majority of clinical colorimetric tests done by the present methods.

The authors' investigation of the degree of precision of colorimetric reactions was aided by a unique photo-electric colorimeter, which was developed after

Fig. 1.

a year's research and careful study of desirable color reading devices. Fig. 1 shows a front view of this instrument which embodies the following desirable features:

First, a multiple set of direct reading scales (see Fig. 3) are provided on a drum behind the slot in the front panel which are calibrated directly against many successive weighed increments of the respective chemicals. This permits the reading of the unknown directly in the desired units without any necessity of intercomparison against a standard solution. (Fig. 2 shows this mechanical structure.) The discussion near the end of this article attempts to confirm the desirability of this altered procedure.

Second, this instrument has the ability to read a specific color density directly and easily within 2 per cent accuracy. There has been substantial evidence of this accuracy through periodic checks against several permanent dry color standards, which indicate no appreciable drift in the calibration in a year.

Third, the unique photo-electric indicator circuit offers a simple means of controlling any possible drift in the initial calibrations after long service.

Fourth, a set of dry color standards of good stability incorporated in the unit may be used conveniently to check the calibration periodically. These color panels are introduced into the light path by means of the little button at the left side of the front panel labeled "Dry Std." (see Fig. 1) which operates a sliding mechanism carrying a set of filters.

Fifth, the electric eye offers an indisputably more precise means of measuring light intensity than the human eye, and in this application it could eliminate

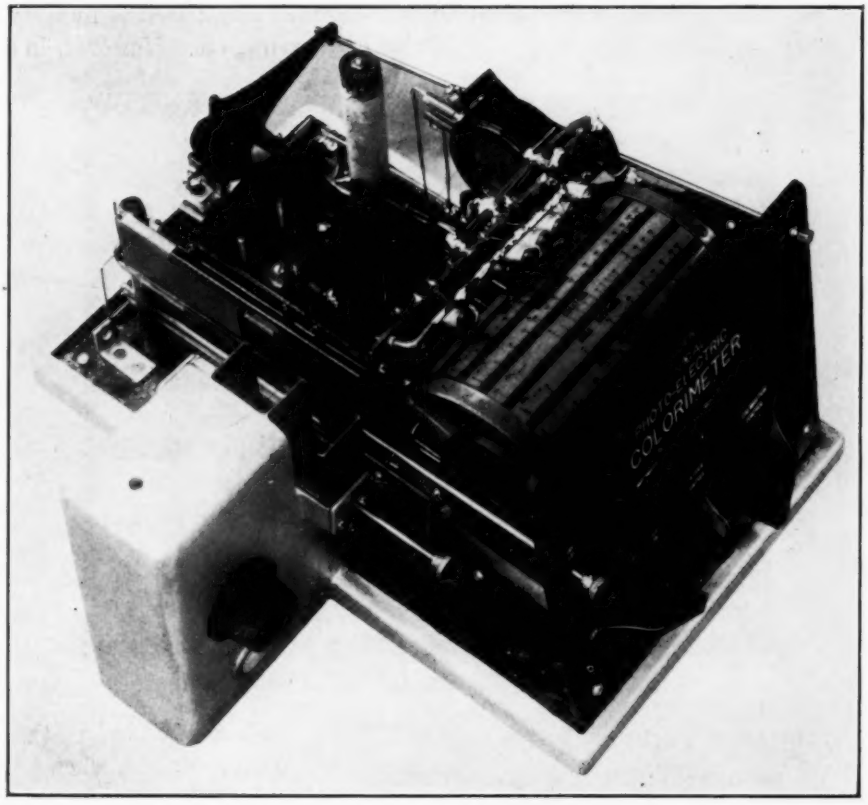

Fig. 2.

another one of those undesirable human elements from a routine scientific measurement.

The fundamental analysis of the present system of the matching of colors in a "Duboseq" type colorimeter resolves itself into an attempt to match the intensity of the stimuli to the brain resulting from the separate excitation of opposite sides of the retina. An analogy of this type of measurement is the determination of the weight of an object by holding it in an extended hand and attempting to adjust a standard weight in the other extended hand which will equal the unknown weight, by comparison of the muscle reaction of each arm. In both the case of the retina and the extended hand, differences in such factors

as initial development, fatigue, etc., greatly influence the accuracy of measurements undertaken with these organs.

The authors anticipate the reluctance of chemists and technicians to abandon the control or standard in these tests. They agree, however, that the control should not be abandoned completely but suggest that with this type of colorimeter the control would be needed only periodically for many routine tests. If we consider for a moment the fundamental purpose of treating a standard solution simultaneously with the unknown, as is the practice, we find that this dual chemical treatment, if it is perfectly similar to the superlative degree, eliminates errors in the reading of the unknown which might otherwise be introduced through variations in treating technic, such as variations in temperature, time, concentration or intrinsic activity of reagents, etc. However, in view

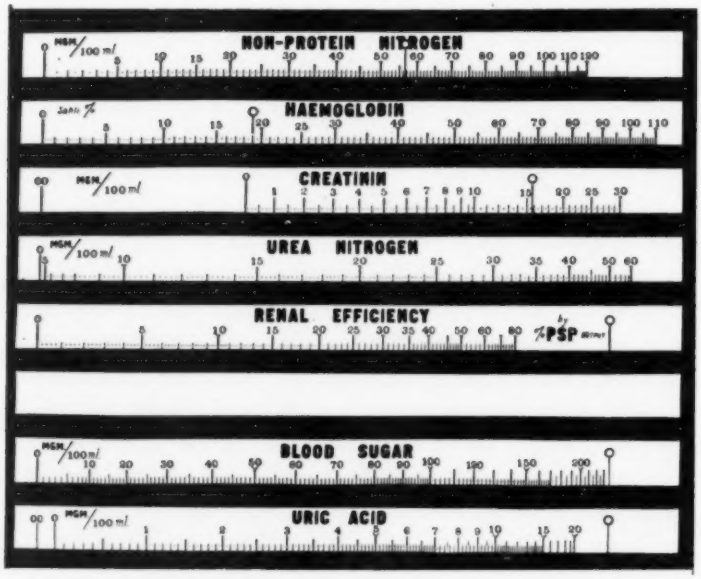

Fig. 3.

of the foregoing discussion of the data of Table I, it should be obvious that the absolute color density of both the standard and unknown solutions, when treated with routine (not absolute) similarity, will usually vary, irrespectively, about 10 per cent above or below the par* color density. Furthermore, if this variance is of the same sign and percentage of magnitude, in both the standard and the unknown, it is certainly a purely accidental coincidence, as it was shown that this variance does not routinely have as close a relation to the treating technic as is generally supposed. Obviously the control for *each* routine test has more often played the rôle of a necessary adjunct to the present comparison colorimeters, rather than being the proverbial guardian of precision.

Since the sign and the magnitude of the variances (from par color density) in the standard and unknown solutions are only remotely correlated routinely, the authors suggest that a reading of the absolute color density of the unknown,

*Par color density is that density which theoretically should be obtained with perfectly standard chemical treatment throughout.

directly in terms of the desired units, yields a result fully as reliable as does the present routine method, and in many cases more accurate: and by reading a routinely prepared standard periodically, a simple percentage correction can be applied to the readings of routine analyses to compensate for deterioration of the reagent. With this correction applied the results, which this direct reading type instrument yields, are definitely more accurate. If this direct reading method were ultimately adopted, the resulting elimination of the control for each test of many types would greatly simplify the routine clinical work in colorimetry.

The authors offer their colorimeter design as the possible adjunct to this suggested modernization which they believe will eventually be universally adopted for routine clinical work.

SUMMARY

1. Some unrecognized errors in present methods of colorimetric determinations have been noted and explained.

2. A new type of photo-electric colorimeter has been described.

3. A modernization in colorimetric procedure is suggested, which offers simplicity and accuracy.

IODIZED OIL*

A Practical Method of Preparation

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FOR many years iodine derivatives of the fatty oils have been known to chemists, but the diagnostic and therapeutic value of such preparations was not generally recognized by the medical profession until about 1921.

According to Sicard and Forestier,[1] the first true iodized oil was prepared in 1856, by Personne, who introduced a small amount of the element into cod liver oil. In 1896, Winternitz produced a chloro-iodized sesame oil known as iodipin, though he gives Hübl[2] (1884) credit as the first to devise a practical and reliable technic for this procedure. In 1901, a good iodized oil, containing 40 per cent iodine but no other halogen, was made by Lafay.[3] This oil is known as lipiodol.

During the last fourteen years, in response to the increasing demand for a suitable iodized oil, numerous methods of preparation have been advocated. In most of the procedures, iodine is made to combine, at the double bond, with unsaturated fatty acid radicals through the intermediate formation of iodine chloride, which in turn liberates hypoiodous acid[4] by a secondary reaction with water. Whether the iodine halide is prepared by decomposition of iodine trichloride, chlorination of iodine in solution by means of chlorine gas[5] or aqua regia,[6] or with the aid of mercuric chloride,[7] the resulting action upon the oil

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is essentially the same. Iodine and its companion halogen are fixed at the unsaturated valences of the double linkage in a ratio that is, in most cases, one to one. Lipiodol is supposed to be made by the action of hydriodic acid on poppy-seed oil. The reaction is undoubtedly similar to that which occurs when erucic acid is converted into monoiodobehenic acid.[8]

Lipiodol is a very satisfactory oil for diagnostic and therapeutic purposes but is expensive and comparatively unstable when exposed to air and light. The iodized rape seed oil (campiodol)[7] is reported to deteriorate only very slowly, but the process of manufacture is somewhat too tedious for those physicians who desire to prepare their own product. Sesame oil, iodized according to the method of Schon and Jacobsen,[5] is said to be unaffected by air, even after exposure for long periods, and the technic is relatively simple though time consuming, because the final product must be aerated for twenty-four hours before it is ready for use.

We are now making an iodized oil in our own laboratory by a convenient and practical method in which iodine trichloride is first employed with the subsequent production of iodine monochloride (iodine chloride) as the intermediate iodizing agent. This procedure was originally reported in foreign literature. We are greatly indebted to Major R. L. Holt, of the Fitzsimons Army Hospital, Denver, Colorado, for first calling our attention to it and for many helpful suggestions concerning other steps in the preparation of the oil.

TECHNIC FOR PREPARING AN IODIZED POPPY-SEED OIL

I. Preparation of Iodine Trichloride.—Place 35 gm. of resublimed iodine crystals in a large, dry test tube immersed for three-fourths its length in water at 15° C. Introduce the free end of a glass tube (leading from the flow meter of a Dakin chlorinating apparatus) clear to the bottom of the iodine container in such a way that the iodine crystals lie loosely around it. Allow the chlorine to flow into the iodine for twenty minutes at a "flow-meter pressure" not to exceed 45 mm. of water. The crystals are rapidly converted into a dark red liquid. As the reaction nears completion, the citron-yellow crystals of iodine trichloride ascend the walls of the test tube. At this point, ice is placed in the cooling bath.

II. Decomposition of Iodine Trichloride With the Formation of Iodine Chloride and Hypoiodous Acid.—To the iodine trichloride add 150 c.c. of distilled water at 15° C. and transfer it to a two-liter flask partially immersed in water at 5° C. As soon as the solution is complete (not more than ten minutes), 100 c.c. of cool, fresh poppy-seed oil are slowly added. Agitate during the introduction of the oil, and continue until all of the iodine chloride has been taken up by the oil. The finished product is a thick, ivory-white emulsion. Allow the preparation to stand for at least two hours.

III. Separation of the Iodized Oil From the Acid-Aqueous Solution.—Pour in 100 c.c. of pure chloroform, shake the new emulsion thoroughly for at least five minutes, and transfer the entire contents of the flask to a 1,000 c.c. separatory funnel. Stratification of the oil and aqueous layers starts rapidly, becoming complete in approximately three hours, though it is perhaps better to wait for six hours.

IV. Removal of Chloroform From the Iodized Oil.—Separate carefully the lower (oil-chloroform) layer from the upper strongly acid, watery layer, which still contains some iodine chloride, place in a 2,000 c.c. evaporating dish which is heated by a boiling water-bath. Within twenty minutes the temperature of the contents of the evaporating dish should be 80° C., at which temperature chloroform is rapidly expelled. At the end of thirty minutes, place an electric fan so that a current of air sweeps continuously across the surface of the liquid, and continue heating, with frequent stirring, for an additional two and one-half hours. The chloroform is eliminated much more rapidly if air is bubbled through the mixture during the entire process of heating.

The appearance of the oil changes several times. For about ten minutes it is a creamy tan while the major portion of the chloroform boils away. At the end of thirty minutes, the color has changed from a pinkish brown to a mahogany brown, then to a pale yellow. During the next two hours, the color becomes and remains a pale amber, the darkening being due to a slight amount of oxidation.

Removal of the chloroform is complete only when the taste of this chemical is absent even when the warm oil is dropped on the side of the tongue.

While still warm, the oil is passed through fine muslin and then through dry filter paper to remove a small amount of resinous material (oxidation products), transferred to 20 c.c. containers which are filled to the brim and covered by an aluminum cap.

Before releasing the oil for therapeutic purposes, each lot is checked by shaking separate portions with water and with a potassium iodide solution and testing the aqueous phase of the first mixture with litmus (free acid), and of the second with starch paste solution (free iodine). No free acid or iodine should be found.

V. The Finished Product.—Prepared by the foregoing method, the iodized oil is a moderately viscous, pale amber, neutral liquid with an oleaginous taste (resembling "cooked" oil) and a specific gravity of 1.20 to 1.28 at 37° C. Its viscosity, compared with water at 37° C., is from 380 to 410 or 260 to 280 centipoises. It has an iodine content of 22 to 25 per cent and a chlorine content of 6 to 8 per cent. The viscosity, gravity, iodine and chlorine content vary with different batches. It contains no free iodine. Little or no color change, and neither free acid nor free iodine can be detected after standing for several weeks in the dark. Slight darkening is observed when the product is exposed for the same length of time to light, but repeated tests still fail to show the presence of free iodine or free chlorine.

As previously stated, the oil contains chlorine; however, its presence is in no way objectionable clinically. On the other hand, we have evidence to indicate that the presence of chlorine actually lends increased stability.

Recently we[9] have described somewhat in detail the clinical use of the oil

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A METHOD OF ELIMINATING BLASTOCYSTIS HOMINIS FROM CULTURES OF ENTAMOEBA HISTOLYTICA*

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THE presence of *Blastocystis hominis* in cultures of *Entamoeba histolytica* interferes greatly with their growth and reproduction. In many instances this organism will alter the nutritional, chemical, and physical state of the culture medium so rapidly that *E. histolytica* can no longer survive. This, perhaps, is one of the most frequent causes of failure to maintain *E. histolytica* in culture.

Various workers in the field of protozoology have recommended the addition of a small quantity of heat-sterilized, dry rice starch to each culture, at the time of inoculation or transfer, to militate against the occurrence, or to control the growth of *Blastocystis hominis*. In the experience of the author this procedure does not rid the cultures of *Blastocystis hominis*, nor does it sufficiently restrict the growth of this organism to the point that it will not interfere with the growth of the amoeba.

Studies on the growth habits of *Blastocystis hominis* show that a pH of 7.4 to 7.8 affords the optimum reaction for its growth, while a maintained pH of 6.8 to 7.0 tends to inhibit its growth. In cultures where the bacterial flora splits the rice starch and the pH is reduced to 6.8 or 7.0, the growth of *Blastocystis hominis* is partially restricted; in cultures containing rice starch where this reduction in pH does not take place, there is no inhibition of its growth.

The influence of various concentrations of neutral acriflavine upon cultures of *E. histolytica* contaminated with *Blastocystis hominis* has been investigated with the following results:

1. *E. histolytica* will live and reproduce in a 1 to 50,000 solution of neutral acriflavine in suitable culture medium.

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2. Such a solution of neutral acriflavine in Boeck-Drbohlav culture medium is toxic for *Blastocystis hominis*, and tends to inhibit its growth and reproduction.

3. If, after treating the cultures on Boeck-Drbohlav medium with 1 to 50,000 neutral acriflavine for forty-eight hours, three successive transfers at twenty-four-hour intervals are made in suitable media in which the pH is maintained at 6.9 to 7.1, then transferred to regular Boeck-Drbohlav medium, the *Blastocystis hominis* will completely disappear from the culture after several transfers and *E. histolytica* will grow unhampered.

In accordance with the above findings, the following method has been devised which has never failed to rid *E. histolytica* cultures of *Blastocystis hominis*. At the time of inoculation, or transfer of all cultures in the subsequent procedure, 0.05 c.c. of a dry, sterile mixture of two parts of powdered rice starch and one part of powdered animal charcoal is added to each culture.

1. Amoebae cultures containing *Blastocystis hominis* are treated as follows: Add sufficient sterile 1 to 10,000 neutral acriflavine in Ringer's solution

Ringer's Solution	Sodium chloride	8.0 gm.
	Potassium chloride	0.2 gm.
	Calcium chloride	0.2 gm.
	Distilled water	1000.0 c.c.

to the supernatant fluid of the culture on Boeck-Drbohlav medium to make a 1 to 50,000 solution. Incubate at 37° C. for twenty-four hours, transfer the culture, repeat the above procedure for another twenty-four hours, and then transfer the culture to lactic acid medium prepared as follows:

2. *Lactic Acid Medium.*—Prepare the following modified Locke's solution and sterilize it in an autoclave at 15 pounds' pressure for fifteen minutes:

Locke's Solution	Sodium chloride	9.00 gm.
	Calcium chloride	0.24 gm.
	Potassium chloride	0.42 gm.
	Sodium bicarbonate	0.20 gm.
	Lactic acid, 1.0 N	0.23 c.c.
	Distilled water q.s. ad.	1000.00 c.c.

With sterile precautions, mix one part of sterile horse serum with seven parts of the cool, sterile Locke's solution. Then add 5 c.c. of the resulting solution to previously prepared sterile egg slants, i.e., the solid media prepared for Boeck-Drbohlav media. Transfers of the cultures on lactic acid medium are made at twenty-four-hour intervals for three successive times and then the cultures are transferred to straight Boeck-Drbohlav medium. There will still be some *Blastocystis hominis* present in the culture, but the organisms will usually all be in a degenerated condition and will completely disappear upon successive transfers of the culture. In case they do not, the entire procedure is repeated, and to date, the second repetition has never failed to eliminate this organism.

A METHOD OF ESTIMATING BOTH BASAL AND EXERCISE CARDIAC OUTPUT ON DOGS*

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IN VARIOUS laboratory studies it would be advantageous to know an animal's cardiac output during exercise as well as at rest. Such data, if reliable, would be useful in experiments involving changes or attempted changes in the cardiovascular system or in estimating physiologic reactions to exercise before and after experimental interference with the animal. Though basal, or resting, cardiac output is readily estimated in the dog by application of the Fick principle, a measure, by similar means, of the exercise cardiac output meets with several obstacles. One must not only find out what the oxygen consumption per minute is while the dog is actually at work but must also obtain samples of blood representing the arterial and venous oxygen content during work. In order that comparisons can be made between various experiments the amount of work done must be ascertainable. Since no standardized method is available the method we use with satisfaction is presented. The usual procedure will be given briefly and then amplified and discussed fully under appropriate headings.

METHOD

Male dogs, found satisfactory by trial for the work, are standardized regarding heart rate, respiratory rate, temperature, blood pressure, and resting cardiac output. The blood pressure is taken by direct femoral artery puncture. The resting cardiac output is obtained by application of the Fick principle. The oxygen consumption is measured by use of a Blalock mask and a closed circuit spirometer with valves. The oxygen content of the right and left heart blood (or femoral artery blood) is measured on a Van Slyke machine.[1, 2, 3, 4]

While the dogs are being thus standardized, they are run on an electrically driven treadmill a little each day until they are accustomed to it. At the same time a special mask, described later in the text, is little by little, on successive trials, fitted over the dog's head until he tolerates it satisfactorily. The dog is then, in a similar manner, accustomed to breathing into an attached closed circuit spirometer while he runs.

The dog having been thus prepared, the actual estimation of exercise cardiac output is done by running the dog about a half hour, at a certain speed, and with the floor of the mill at a certain slope, during which time several oxygen consumption curves are taken over five- to ten-minute periods. At the end of half an hour the dog is rapidly removed from the mill and arterial and right

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heart blood samples are taken. One pair of samples are taken within one-half minute of cessation of exercise usually and two other pairs at intervals of about one minute each. As the first two pairs of samples usually agree closely it can be assumed usually that the first arterial and venous samples represent, with fair accuracy, the arterial and venous oxygen values when the animal is at work. By use of the Fick principle the exercise cardiac output is calculated from these figures. A check of the approximation of the oxygen content of such blood samples to the actual arterial and venous oxygen content at work is carried out when all studies on a dog are completed. Samples of right and left heart blood are taken during actual exercise, both while oxygen consumption is being measured, and while the mask is not worn. A close agreement is found to exist between the figures obtained by the two methods.

DISCUSSION OF METHOD

Preparation of Animals.—It is worth while to choose carefully the animals used in these experiments. Male dogs only can be used because the females have an unstable cardiac output. A dog anxious for work, intelligent and yet of a passive disposition, proves excellent. By a brief trial consisting of several fittings of the Blalock mask and the special mask, a few runs on the treadmill, and several trials at resting oxygen consumption, it can be readily judged whether a dog is likely to be satisfactory. Suitable dogs are accustomed to lying quietly in the dorsal position on an animal board, to the Blalock mask connected with the spirometer, to cardiac and arterial puncture, and to running on the treadmill. When the dogs are quite used to the treadmill the special mask is put on little by little, on successive trials, until the animal runs quite nonchalantly with the mask completely adjusted. Because it is found that often, on the first few occasions that the mask is connected with the spirometer, the dog is slightly disturbed, gradual initiation in this maneuver results in the dog rapidly becoming used to the procedure.

Basal Output.—The basal cardiac output is calculated in the usual way except that we prefer to take samples of right and left heart blood (or femoral artery blood) several times during the course of one of several resting oxygen consumption estimations. An accurate judgment can thus be made of the reliability of the test. To check our results further the circulation rate is tested from time to time, during a basal oxygen consumption test, by the cyanide method. This, with a count of the pulse and respiratory rate, forms a useful guide to the animal's true state of rest.

Exercise Output.—To explain more fully the rationale of the method which we feel is satisfactory for obtaining a figure accurately representing the cardiac output in exercise, the method will be discussed and amplified under the headings: The Oxygen Consumption Estimation; Estimation of the Arteriovenous Oxygen Difference; and Estimation of Work Done.

The Oxygen Consumption Estimation.—An estimate of the oxygen consumption of exercising dogs has been attempted by some by taking the oxygen consumption measurement immediately after the cessation of work. A variety of masks have been used, some of which fitted the snout tightly like the Blalock

mask, while others fitted over the head. The former type will never fit a dog satisfactorily that is breathing in a manner corresponding to its exercise respiratory volume. In any event, no matter what mask be used, the method can be shown to be inaccurate. Certain dogs maintain a high oxygen consumption for some minutes after exercise with long continued excessive ventilation and

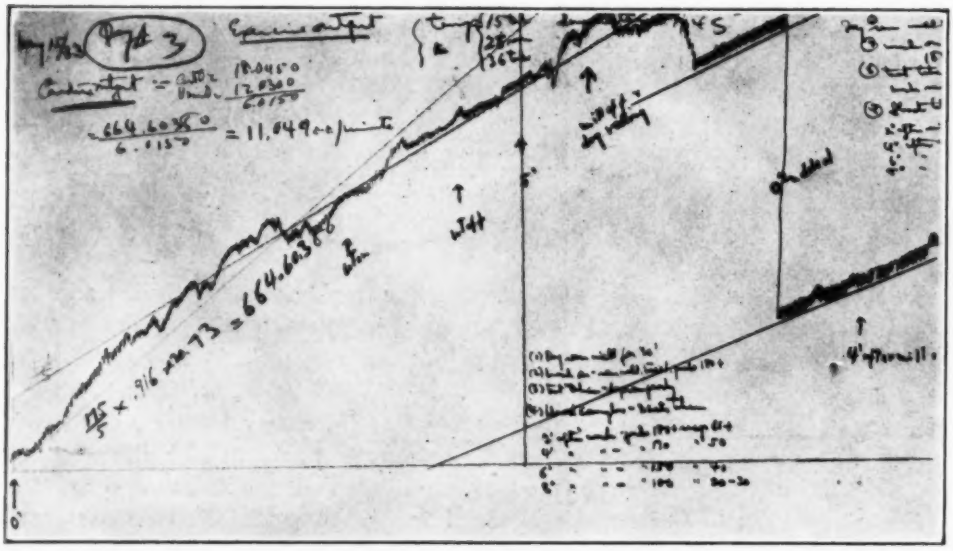

Fig. 1.—The oxygen intake curve of a very active dog. Its oxygen intake remained high for some minutes after the treadmill was stopped and the dog was made as comfortable as possible.

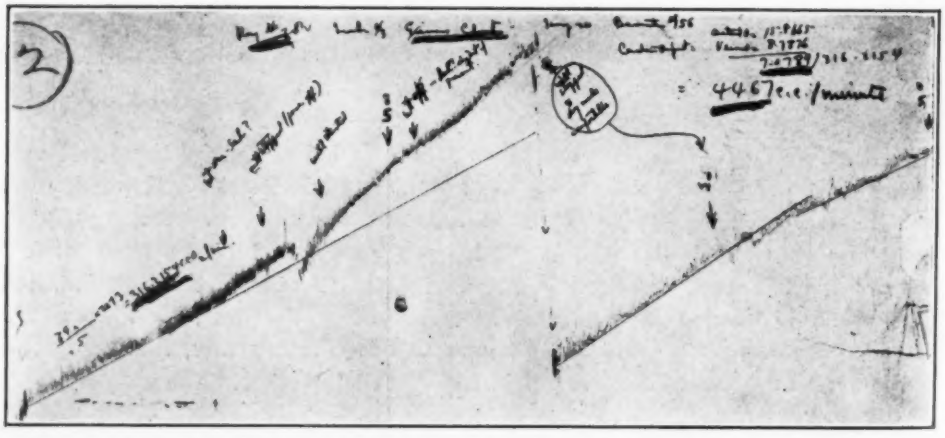

Fig. 2.—The oxygen intake curve remained high for this dog after cessation of work but the arteriovenous oxygen difference increased. This lowered the figure for exercise cardiac output to 4467 c.c. per minute. The exercise cardiac output was usually about 10,000 c.c. per minute when oxygen intake and blood sample were taken while the animal was actually running.

panting (Fig. 1). An oxygen consumption curve, taken with a suitable mask on such dogs after work, would sometimes be useful but would not represent reliably the actual exercising oxygen intake figure. Moreover, though oxygen intake on such a dog remains near the exercise level after work for some time, the venous oxygen content may change rapidly because of slowed circula-

tion and, by altering the arteriovenous oxygen difference from its exercise level, may produce a change in the figure for the cardiac output. Fig. 2 illustrates an instance where oxygen intake remained high for some minutes after cessation of work but the venous oxygen content fell rapidly to increase the arteriovenous oxygen difference and diminish the figure for cardiac output. On the other hand, many dogs exhibit an exercising oxygen consumption curve which very rapidly falls toward the basal level after exercise. These dogs can usually be fitted with a mask that fits either the snout or the head tightly, for they pant very little. But the curve obtained in this way is of negligible value. Fig. 3 illustrates how an oxygen consumption of 282 c.c. per minute during

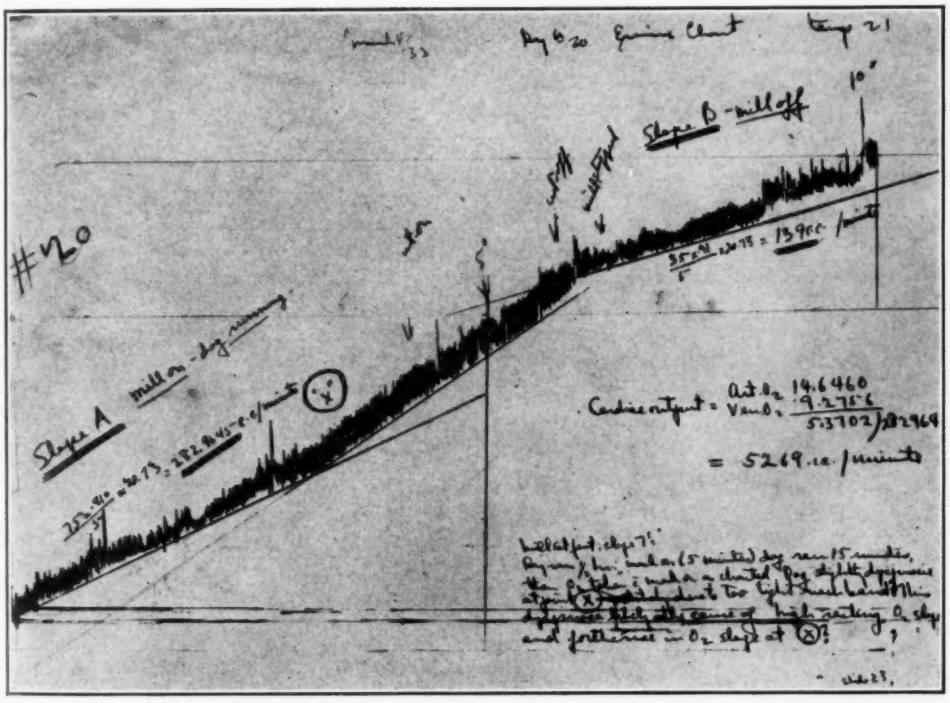

Fig. 3.—The first six minutes of oxygen intake. Slope marked "Slope A" is taken with the dog running. "Slope B" is much lower and commences when the dog stops running.

exercise, represented by the first six minutes of the curve and marked "slope A," changed rapidly on cessation of work to an oxygen consumption of only 139 c.c. per minute, represented by the last four minutes of the curve and marked "slope B." Fig. 4 illustrates a typical exercise consumption curve taken on a dog actually at work and the oxygen consumption curve on the same animal taken immediately after the cessation of work. The former curve represents an oxygen consumption of 337 c.c. per minute while the latter represents an oxygen consumption of 97 c.c. per minute.

Therefore it seems more accurate to measure the oxygen consumption while the animal is actually at work. Intertracheal cannulas may of course be used but are liable to cause the animal discomfort, require much care, and in many

dogs during exercise may leak or become plugged by an overproduction of mucus. For these reasons several masks which a dog could wear while running were designed. Lightness, visibility, ease of adjustment, and air tightness were the prime requisites. The mask which proved most suitable is readily and very cheaply made as follows:

A celluloid cylinder is rolled of suitable dimensions just to fit over the dog's head. Ordinary celluloid or cleaned x-ray film may be used, the edges be-

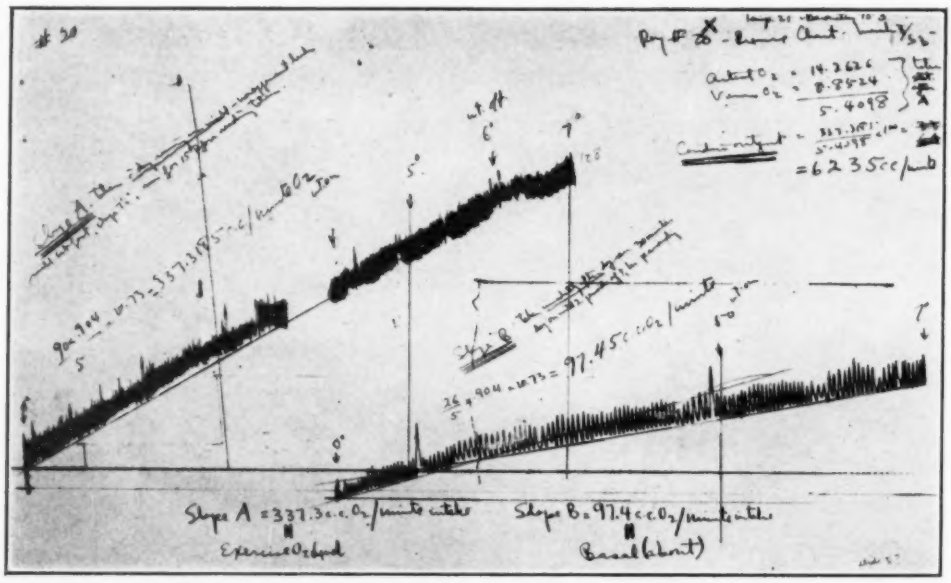

Fig. 4.—"Slope A" illustrates the high oxygen intake while a dog runs. "Slope B" illustrates a very much lower oxygen intake immediately after exercise was finished.

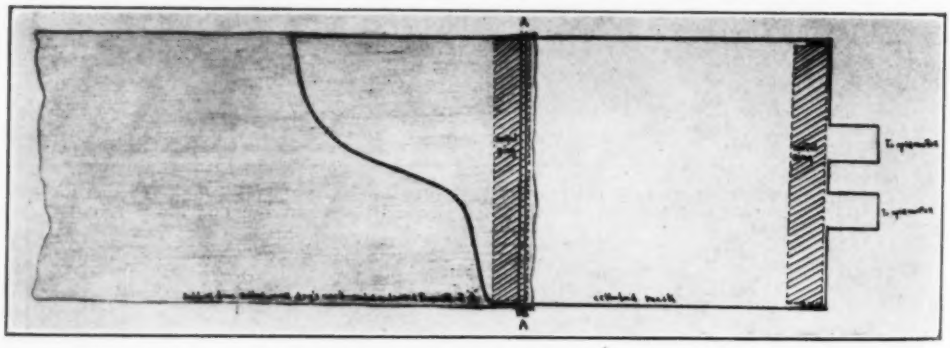

Fig. 5.—Diagram of mask.

ing easily sealed with acetone or by the application of an adhesive such as "Ambroid." The cylinder's length is such that with its one end several inches in front of the snout, the other end comes just behind the ears. A light metal ring supports the front of the cylinder while toward the back another ring lends support and also has a cord fixed to it which can be fastened behind the dog's neck to hold the mask in place. The front of the cylinder is fitted air tight with either a light tin or celluloid disc equipped with two centrally placed

tubes designed to fit exactly the ends of the spirometer tubes. The caudal end of the cylinder is shaped in its upper half to project a little over the back of the dog's neck while the under half is cut in a curve to fit the dog's throat. A piece of light rubber dam made in tubular fashion is tightly bound and sealed to the caudal end of the mask and can be rolled over the dog's shaven neck which it fits snugly. It is further held to the neck by several wide rubber bands carefully adjusted to avoid undue pressure, especially on the veins (Fig. 5).

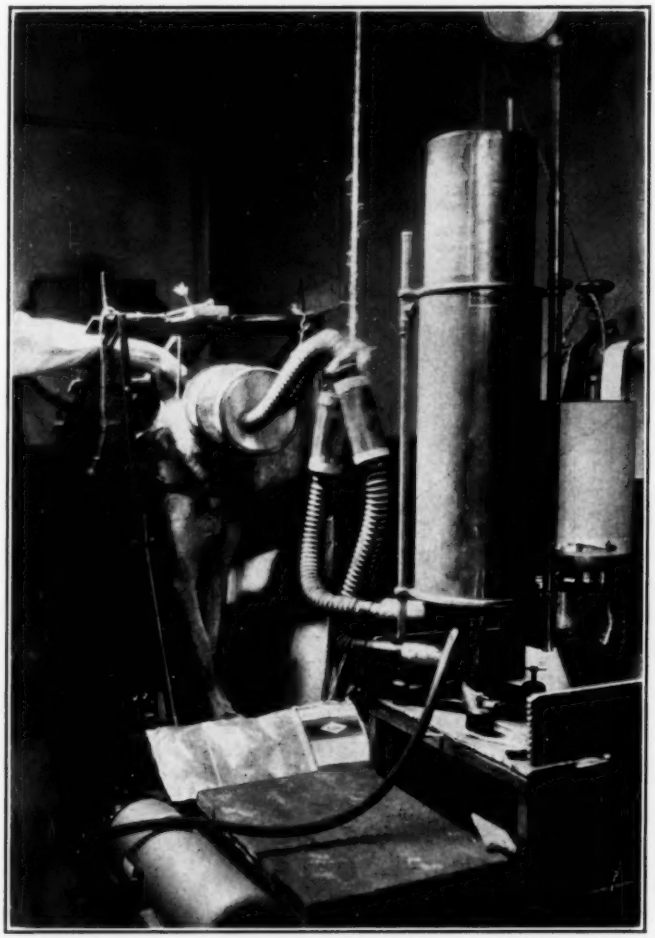

Fig. 6.—Photograph of dog on treadmill wearing mask which is joined by flexible tubing containing flutter valves in celluloid housings to a closed circuit type spirometer.

Such a mask has proved very satisfactory and surprisingly leak proof in our hands. Each experiment is tested for leakage. Small leaks must in any event prove negligible on account of the very large oxygen intakes registered. Dead space in the mask is also negligible for the same reason and because the dog's head leaves little space actually unoccupied. In warm weather humidity in the closed circuit used occasionally makes the animals uncomfortable. Removal of the apparatus to a cold room corrects this difficulty.

The spirometer used is of the closed circuit type with valves. The dead space in the tubing is rendered negligible by placing the valves near the mask end of the tubing. The tubing is suspended from a point near the valves so that the dog is not inconvenienced in any way (Fig. 6). With this arrangement a blower is found unnecessary. Besides, a blower might readily produce leakage, by increasing the pressure in the system. The oxygen curves are taken over periods of from five to ten minutes at the end of the exercise period. Sometimes several curves are taken at different parts of the exercise period to check the variability of oxygen consumption. It is found that after exercise has been

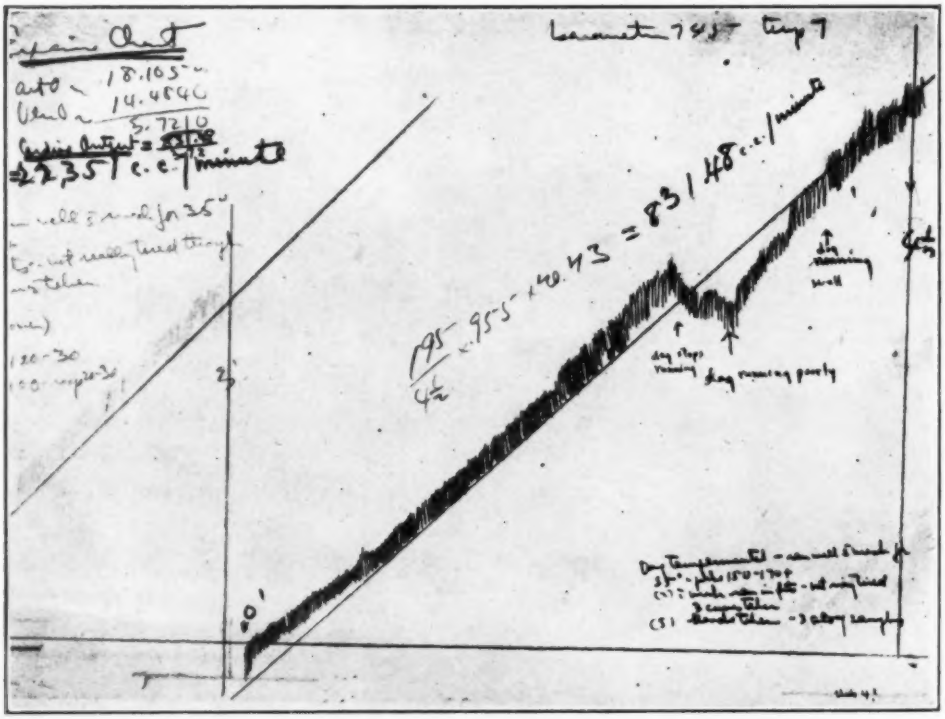

Fig. 7.—The steep oxygen curve obtained while a dog runs usually changes rapidly with the amount of work done so that from the curve one can judge the performance of work.

well started oxygen consumption maintains a fairly level course. In fact the curves are an excellent indication of the work being done, for usually any change shows in the oxygen consumption curve quickly and markedly (Fig. 7). For each dog the amount of oxygen used for a constant amount of work is very similar in a series of tests. The oxygen intake is recorded and measured in the usual way on an extra tall kymograph cylinder in order to allow for the high oxygen curve registered.

Estimation of the Arteriovenous Oxygen Difference.—The estimation of the arteriovenous oxygen difference in exercising animals would be done ideally by direct puncture of the left and right heart. This procedure is not feasible when valuable experimental animals are being used on account of the danger of cardiac tamponade, the result of nicking a coronary artery. However, it is

found that if three sets of femoral artery and right heart blood samples are taken immediately work is finished, at intervals of about a minute, an idea can be had of how closely the first set of samples approximates the actual exercising arterial and mixed venous oxygen figures. Accordingly the following technic is followed: A sample of femoral artery and of right heart blood is usually easily obtained within the first half minute of stopping exercise, the dog being rapidly removed from the treadmill and placed in the dorsal position on an animal board. This first pair of samples usually indicates an arteriovenous difference of 3 or 4 c.c. of oxygen per 100 c.c. The venous oxygen content usually falls a little in the second pair of samples to give a slightly increased arteriovenous difference. The third pair of samples, often taken two or three minutes after work has ceased, usually gives a much greater arteriovenous difference due to lowered venous oxygen content. There is presumably, then, no great change between the arteriovenous oxygen difference during exercise and during the first minute of rest following exercise. By this procedure, however, the animal is put suddenly at comparative rest and simultaneously removed from an atmosphere of variable oxygen tension, but still one which might be of higher oxygen tension than that of atmospheric air. Since both these factors are possibly capable of changing the arterial and venous oxygen relationship a check of the results is made on each dog when its experimental usefulness is completed, and death caused by accidental tamponade is of less consequence. Samples of right and left heart blood are taken while the dog is running and the oxygen intake is being measured, and while the dog is running free of the mask. The figures for the arteriovenous oxygen difference obtained in this way are found to agree closely with an average of the figures for the arteriovenous oxygen difference of the first arterial and venous samples drawn from the same dog immediately after exercise.

It is found that each dog requires a few tests before an idea can be had of its usual reaction to exercise as seen in arterial and venous oxygen estimations. In this series of experiments, in the majority of dogs during moderate exercise, the venous oxygen content rises somewhat, the arterial oxygen content changing either way only slightly. Individual dogs vary to only a small extent from time to time in their arteriovenous blood oxygen difference during exercise though occasionally larger variations do occur (Figs. 8 and 9). Such changes seem due to alterations in circulation time. Because of such variations, and because the rise in the venous oxygen content during exercise in the majority of the dogs is apparently no artefact, circulation time is frequently taken by the cyanide method. As a rule dogs that show a smaller arteriovenous oxygen difference during work than at rest have a very short circulation time while exercising. Dogs that during exercise show an increased arteriovenous oxygen difference over that at rest have a long circulation time at work. This small arteriovenous oxygen difference in exercise seems due to such an increase in circulatory rate in response to exercise that, though oxygen utilization in the tissues increases, the relationship between circulation rate and oxygen utilization is such that venous blood oxygen is not usually reduced but is frequently

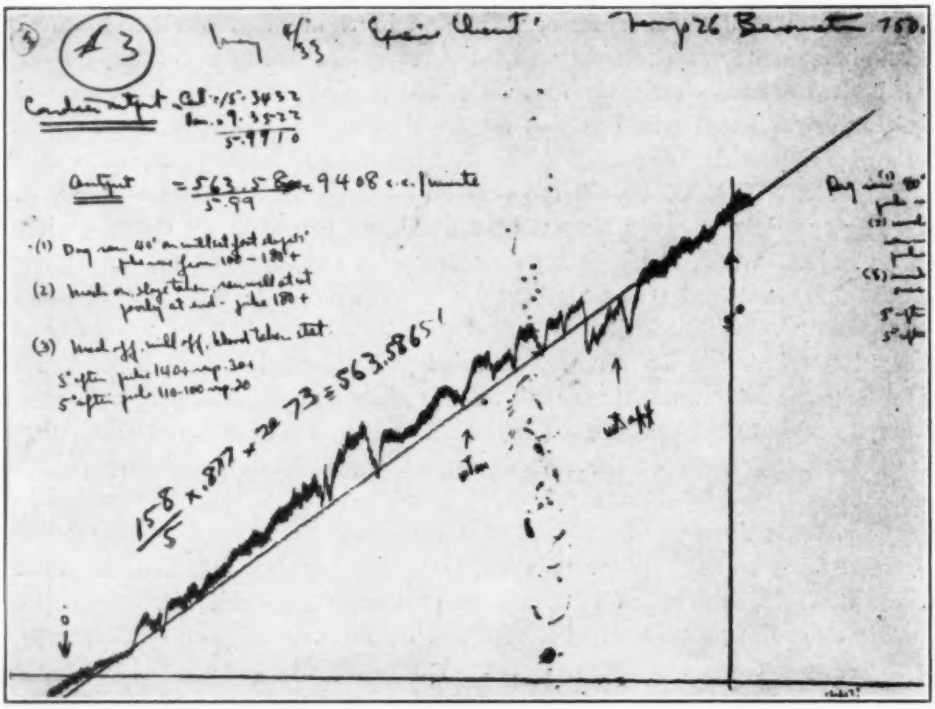

Fig. 8.

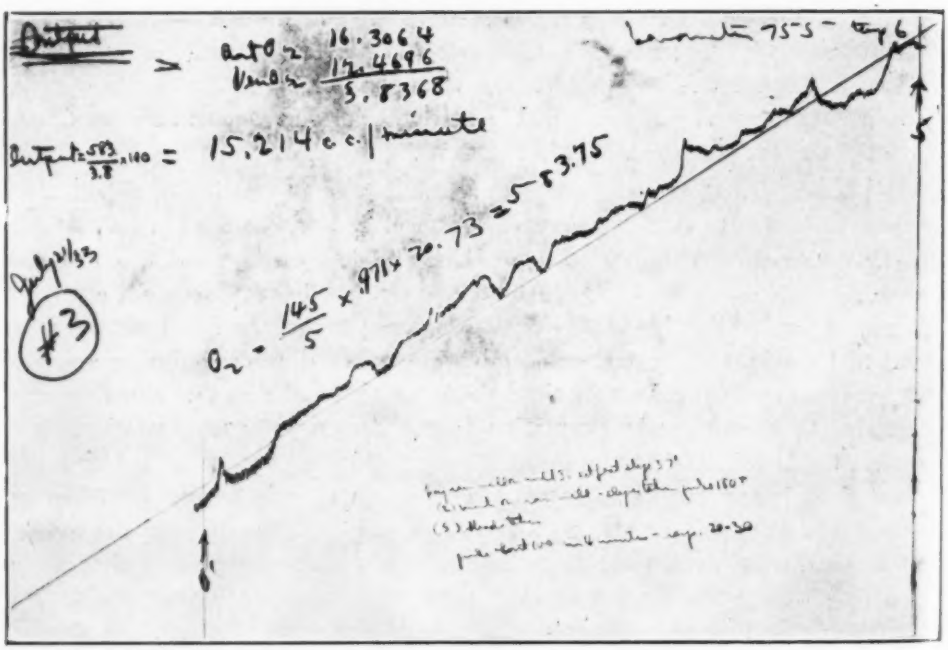

Fig. 9.

Figs. 8 and 9.—Dog 3, May 4, 1933, used 563 c.c. oxygen per minute but because the arteriovenous oxygen difference was 6 c.c. per hundred the cardiac output was only 9,408 c.c. per minute. July 21, 1933, the oxygen intake was 583 c.c. per minute, the arteriovenous difference was 4 c.c. and the cardiac output was 15,214 c.c. per minute. A very similar amount of work was done on each occasion. Such variations occasionally occurred making it necessary to carefully study each dog's individual reaction to exercise so that the average reaction could be known and deviations recognized.

higher than at rest. Figs. 10 and 11 illustrate the constant exercise output figures arrived at by the method outlined above.[3, 5-10]

Estimation of Work Done.—The amount of work done is best kept at a moderate level where a group of dogs are to have a series of similar experiments

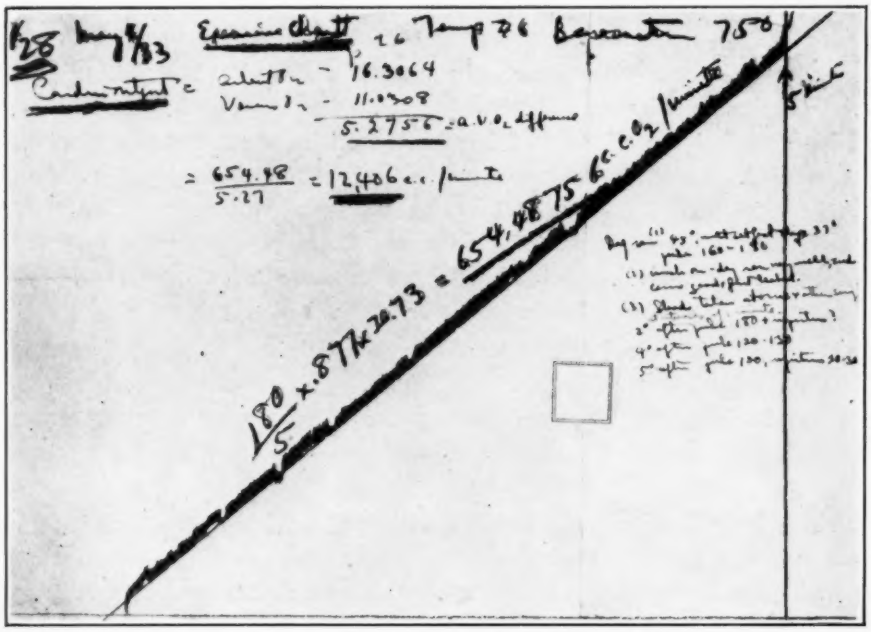

Fig. 10.

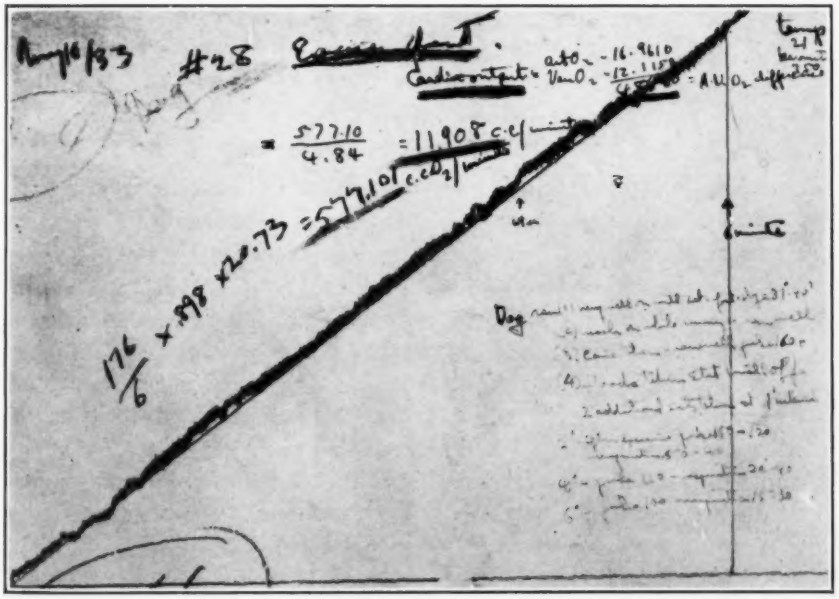

Fig. 11.

Figs. 10 and 11.—Dog 28 gave an exercise cardiac output of 12,406 c.c. per minute May 4, 1933, and May 10, 1933, an output of 11,908 c.c. per minute. A very similar amount of work was done on each occasion. This close correspondence between the tests was usual.

done on them and their cardiac responses compared. Severe exercise, though it gives an estimate of the maximum amount of work the dog is capable of, entails a training factor which is changed by periods of rest such as follow operations. The amount of exercise used in our particular experiments has proved severe enough in any event to give cardiac outputs four to six times the resting output, sufficiently high that any change in the ability of the heart to perform work is readily recognizable. This increase in output is also great enough to offset any of the small errors involved in the method. The time period is kept to one-half hour so that the effects of fairly hard work over a relatively short period are produced. Longer periods of time tend again to train a dog, thus changing from time to time the reaction to exercise of the various functions studied. Other factors in favor of moderate work are a fairly constant reaction from trial to trial, no great variation in temperature with accompanying metabolic changes and no undue disturbance to the animal.[3, 5, 9, 11, 12]

The treadmill used is electrically driven and is run at a speed of about 70 meters per minute with a slope of 37 degrees. The amount of work may be interpreted in terms of energy expended per kilogram by using the formula $E = (0.6 \times a \times \text{Cos } 37^\circ) + (3 \times a \times \text{Sin } 37^\circ) = a \times 1.8$ where "a" represents the distance run at a 37° slope.[11]

There is a slight error in the calculation of work done because it is found safest to hold the dog in the mill with a leash. Though some dogs will usually run without a leash others tend to lag. The leash is fastened to a comfortable harness on the dog and to a suitable crossbar on the mill above and just ahead of the dog's resting position. Dogs that run well expend energy as governed by the speed and slope of the mill. But dogs that persistently have part of their weight carried by the leash are discarded. Careful note is kept during each experiment of the dog's behavior and performance of work, of the number of minutes taken for pulse, respiration, and blood pressure to return to normal levels, and of the dog's general appearance after work.

Though it is true that a dog's performance of work varies from time to time, for comparative purposes there is a close enough correspondence between the amounts of work done to justify comparisons between the cardiac responses of a dog from test to test. Tables I and II list the various data collected on two dogs of a series that were being studied to test the effects of coronary artery occlusion on dogs that had had previous stellate ganglionectomy. Table III represents the data on dogs that had not been subjected to stellate ganglionectomy before coronary artery ligation. The basal and exercising cardiac outputs of Tables I and II are fairly constant, with few wide variations, and support the reliability of this method of obtaining cardiac output at rest and during exercise. Further support is lent this view in that the cardiac outputs of all of the other group of dogs, represented by the data of Table III, show a fairly marked change after occlusion of the coronary artery. Apparently variations in cardiac response to exercise are easily discernible. The variation between the two groups of dogs noted in the altered cardiac output, both at rest and during exercise, is confirmed by clinical observation.

TABLE I

Typical figures obtained by use of the described method for basal and exercise cardiac output in a series of dogs that had stellate ganglionectomy done before coronary artery ligation was done. Clinically and by tests no great change in health occurred. Note the uniformity of the figures for output.

DOG NO.	WT. KIL.	PULSE RECOVERY REST	PULSE RECOVERY WORK	PULSE RECOVERY TIME	B. P. REST	B. P. WORK	RESPIRATIONS RECOVERY REST	RESPIRATIONS RECOVERY WORK	RESPIRATIONS RECOVERY TIME	O_2 CONSUMPTION C.C./MIN. REST	O_2 CONSUMPTION C.C./MIN. WORK	BLOOD O_2 ART. VEN. REST	BLOOD O_2 ART. VEN. WORK	ART. VEN. O_2 DIFFERENCE REST	ART. VEN. O_2 DIFFERENCE WORK	ART. O_2 C. P.	CARDIAC OUTPUT REST	CARDIAC OUTPUT WORK	E. C. G.	HEART SIZE
60/33	13.0	100	150	2′	85-135		16	28	2′	199.58		19.45-15.36		4.1		20.2	5129			
		90	200	5′	90-120		30	60	5′	149.75		20.83-11.38		9.5			1584		Dec. 7	March
					80-125					113.21		19.52-12.64		6.9		23.7	1644			7.5
						110-165					373.55		15.68-12.28		3.4			10.989		April
	12.9					105-170					487.23		18.31-14.14		4.2	21.8		11.600		7.5
						110-170					277.67		17.93-13.32		4.6			6.031		
		Stellate ganglionectomy																		
					90-130															
	15.2	80	150	6′	90-120		20	60	6′	164.0		22.9-16.4		6.5			2529			
						105-165				108.0		16.5-13.1		3.5		24.6	3144		March	May
						103-170				185.3		22.1-16.3		5.8			3209		30	7.5
										132.9		22.7-17.9		4.6		26.5	2769			
										116.0		18.2-11.9		6.3			1861			
											540.43		20.7-15.9		4.8			11.259		
		Coronary ligature									490.8		18.2-14.8		3.4			14.435		
		90	170	5′	90-120		30	60	5′	137.9		20.7-14.6		6.1			2259			May
					80-130					129.0		22.3-17.5		4.8		23.4	2666		June	7.5
	14.2					105-175				177.0		19.3-13.3		6.0			2932		3	June
						110-170				96.8		19.4-15.1		4.3		24.1	2211			7.5
										137.0		18.7-13.4		5.2			2596			Aug.
										162.0		21.1-14.3		6.7			2425			7.5
											437.0		19.5-13.4		6.1			7.751		
											547.0		22.3-17.5		4.8			11.396		
											402.0		22.7-19.1		3.6			11.166		

TABLE II

Typical figures obtained by use of the described method for basal and exercise cardiac output in a series of dogs that had stellate ganglionectomy done before coronary artery ligation was done. Clinically and by tests no great change in health occurred. Note the uniformity of the figures for output.

DOG NO.	WT. KG.	PULSE RECOVERY REST	WORK	TIME	B. P. REST	WORK	RESPIRATIONS RECOVERY REST	WORK	TIME	O_2 CONSUMPTION C.C./MIN. REST	WORK	BLOOD O_2 ART. VEN. REST	ART. VEN. WORK	ART. VEN. O_2 DIFFERENCE REST	WORK	ART. O_2 C. P.	CARDIAC OUTPUT REST	WORK	E. C. G.	HEART SIZE
3	13.0	100	180	4′	90-140		20	50	3′	112.0		17.9-12.1		5.8		19.3	1931			
					85-140					95.6		14.6- 9.1		5.5			1738		Feb.	March
										113.3		17.5- 9.2		8.3		23.7	1365		1	29
										110.8		17.8-11.7		6.1		21.4	1816			
	12.0					105-160					373.5		16.1-11.2		4.9			7622		8.5
						100-170					497.5		14.5-10.8		3.7			13446		
											462.3		14.1-11.2		2.9			15941		
		Stellate ganglionectomy April 4, 1933																		
	12.7	70	150	6′	78-130		20	60	3′	108.5		14.0- 8.6		5.4		20.2	2009		April	May
					80-135					183.8		19.9-12.5		7.4			2485		7	
										152.9		16.2-11.6		4.6			3323			8.5
										126.6		16.2-10.1		6.1			2078			
	12.2	100	180	6′		100-165					563.5		15.3- 9.3		6.0			9391		
						105-175					664.6		18.0-12.0		6.0			11076		
											324.9		19.6-15.7		3.9			8330		
											579.0		16.4-12.5		3.9			14846		
		Coronary ligature May 23, 1933																		
										82.7		16.8-12.2		4.6			1797			May 23
	12.2	90	160	5′	100-130		20	60	4′	129.5		15.0-10.7		4.3		19.6	3001			May 24
					90-140					73.9		15.3-12.9		2.4			3079			May 25
										149.5		16.9-10.9		6.0			2491			May 28
										131.0		16.8-11.5		5.3		22.8	2452			Aug. 15
										128.4		18.4-11.5		6.9			1860			8.5
						110-160					433.0		12.9-10.2		2.7			16037		
						108-170					391.0		18.4-15.5		2.9			13482		
											583.7		16.3-12.4		3.9			14948		

TABLE III

Typical figures obtained by use of the described method for basal and exercise cardiac output in a series of dogs that had coronary artery ligation done without stellate ganglionectomy being performed. Clinically the dogs seemed less active after operation. Note the high basal output and reduced exercise output.

DOG NO.	WT. KG.	PULSE RECOVERY REST	PULSE RECOVERY WORK	PULSE RECOVERY TIME	B. P. REST	B. P. WORK	RESPIRATIONS RECOVERY REST	RESPIRATIONS RECOVERY WORK	RESPIRATIONS RECOVERY TIME	O_2 CONSUMPTION C.C./MIN. REST	O_2 CONSUMPTION C.C./MIN. WORK	BLOOD O_2 ART. VEN. REST	BLOOD O_2 ART. VEN WORK	ART. VEN. O_2 DIFFERENCE REST	ART. VEN. O_2 DIFFERENCE WORK	ART. O_2 C. P.	CARDIAC OUTPUT REST	CARDIAC OUTPUT WORK	E. C. G.	HEART SIZE
247	19.0	100	180	4-7′	80-150		25	60	7′	147		22.0-18.4		3.6			3972			
					75-140					141		23.6-17.1		6.5		24.5	2169		June 3	June 3
	17.6				75-145					118		23.5-16.7		6.8			1735			8.0
						98-170					330		18.3-13.4		4.9			6734		
						95-168					579		20.8-16.0		4.8	24.0		12020		
						98-170					434		17.3-13.4		3.9			11128		
		Coronary ligature June 14, 1933																		
	15.8	95	160	4′	78-140		25	60	4′	157.3		20.5-16.2		4.3			3658			June 14
		90	150	4′	78-140					184.0		19.0-13.9		5.1		22.8	3607			June 15
										184.0		17.3-12.0		5.3			3471			8.0
											542		22.4-17.6		4.8			11291		July 15
											442		22.4-12.9		9.5			4652		8.0
											482		18.8-12.5		6.3			7650		
											482		18.1-12.9		5.2			9296		

SUMMARY

A method of estimating the basal and exercise cardiac outputs on dogs is given. A discussion of the various difficulties encountered and of the various means of checking estimations is gone into.

A mask we found altogether suitable for obtaining oxygen intake on dogs while at work is described.

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AN AUTOMATICALLY RECORDING COLONY COUNTING APPARATUS*

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AN INCREASE in the accuracy of bacterial plate counts can be brought about by eliminating the causes of physical and mental fatigue of the individuals making the counts. The strict attention required and the effort expended in counting and recording large numbers of bacterial colonies cause rapid tiring of the operator, resulting in a decrease in his accuracy and productiveness. The monotonous nature of the work is furthermore conducive to lapses of memory which not infrequently result in errors.

These drawbacks could be overcome and the speed of counting increased considerably were it possible to free the operator of the mental strain incident to counting the colonies. Attention could then be directed primarily to the detection of bacterial colonies rather than to their enumeration. In each of the counting devices of Stewart,[1] Buck and Swenarton,[2] Buck,[3] Schacht and Robertson,[4] Graf[5] and others the objective has been primarily an increase in the definition of bacterial colonies by improving the lighting or magnifying systems. There is slight hope of further increasing the accuracy of plate counts by improving the methods for visualizing the bacterial colonies.

With the above factors in mind, an apparatus has been devised by me for automatically recording the number of colonies in a Petri dish, and is described below. Following the completion of this equipment a search of the literature showed that Robinson[6] in 1930 had described an apparatus for this purpose, which consists of a marking pen so constructed that by pressing it against the glass back of a Petri dish, over a colony, a mark is left on the glass and an electrical contact is closed. This operates a magnetic counter which automatically records the number of the colony.

Artifacts in the medium or imperfections in the glass may interfere to a considerable extent with counting by such a method. Duplicate counts of certain colonies are not infrequently made, while still other colonies remain uncounted, especially when they are closely spaced or are growing in a thick layer of medium. Such a method furthermore renders difficult the use of counting charts. More accurate results should be obtained were the cover of the Petri dish removed and the count made by actually touching and simultaneously obliterating each individual colony. This procedure could be used since in milk and water work it is seldom necessary to study the bacterial colonies after they have been counted. Such a procedure would decrease the possibility of skipping some colonies and counting others more than once. Moreover, counts obtained by such a method could be readily checked since each colony actually counted would be plainly marked. The automatic colony

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counting device described below is so designed that each individual colony must be separately touched in order to be recorded.

The operating mechanism is enclosed in a substantial housing, through the metal top of which is cut a circular opening, slightly larger than a Petri dish, which serves to hold the latter. A removable cover glass is placed a short distance below this opening, and beneath this is placed a ruled glass counting plate which can be obliquely lighted from below by means of magnetically shielded electric lights so as to render visible the bacterial colonies. The metal top plate of the apparatus is connected through a high resistance to the 110 volt lighting circuit. If direct current is used, this plate must be connected

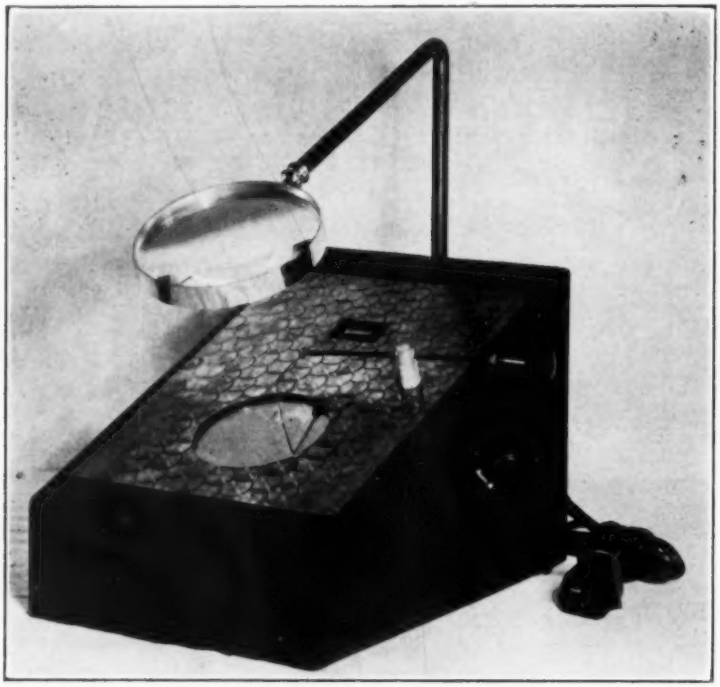

Fig. 1.—The recording colony counter, shown with the six inch magnifying glass in position.

to the positive input terminal only. The operator's hand must be in continuous contact with this plate while counting colonies.

An electrical connection is made between the agar or other nutrient medium and the recording mechanism by means of a removable clip which is connected through a high resistance to the grid of a vacuum or grid glow tube. The plate of this tube is connected to a sensitive relay which operates a magnetic counter. The magnetic relay can be dispensed with by substituting a Thyratron tube for the vacuum tube, but this is not recommended.

An electrical connection between the nutrient medium and the metal housing on which the hand of the operator rests is made by means of a metal electrode which is held in the hand of the operator. This electrode has an interchangeable tungsten tip, a blunt point being used for large colonies and

a sharp point for small colonies. It is readily manipulated since it is unattached to any part of the apparatus.

When a colony is touched the recording mechanism is operated by means of the imperceptible current which passes through the hand, electrode, agar, and high resistance to the grid of the vacuum tube. This minute current causes the grid to become positively charged, permitting the passage of a small plate current which operates the relay and trips the magnetic counter. When the electrode is withdrawn from the agar the grid again becomes negatively charged, stopping the flow of plate current and opening the relay. This resets the counter in readiness for the next operation.

The apparatus emits a distinct click each time a colony is touched, hence there is no danger of unknowingly counting a colony or of not knowing when a colony has been counted. Contacting a colony for one-twentieth of a second suffices to operate the recording mechanism. If, due to unsteady

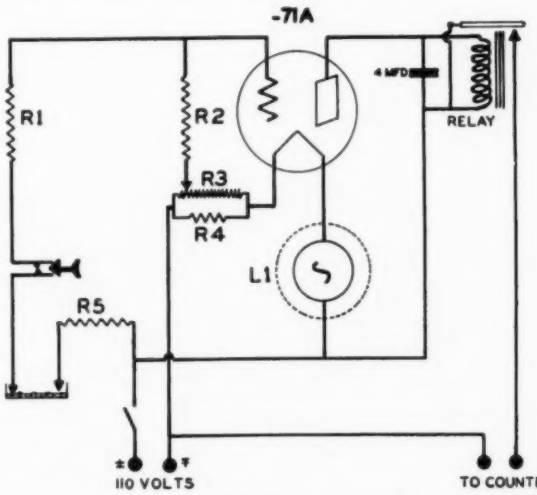

Fig. 2.—Wiring diagram of the colony counter. R1-R5, overload protective resistances; R2, grid leak resistance; R3, grid potentiometer; R4, filament resistance; L1, 25 watt magnetically shielded show-case type light.

hands, a colony is accidentally contacted twice the resulting overcount can be compensated for by means of a circuit opening switch, the use of which permits the operator to touch the next colony without causing it to be recorded.

The apparatus is equipped with a large magnifying lens by means of which the bacterial colonies can be observed. There is adequate room between the Petri dish holder and the lens for the hands and counting electrode.

The apparatus is illustrated in Fig. 1 and the wiring diagram is shown in Fig. 2. The relay hook-up operates on both direct and alternating current, but the magnetic counter operates on only one type of current, which must be specified when securing this portion of the apparatus. The 4 m.f.d. condenser shown is not needed if the apparatus is operated on direct current.

SUMMARY

A counting apparatus operated by a vacuum tube is described, by means of which bacterial colonies can be counted and recorded more rapidly and

accurately and with considerably less fatigue than by other methods. When a bacterial colony is touched with a free electrode held in the hand, a mechanism is operated which automatically records the number of the colony on a magnetic counter.

NOTE: This apparatus may be obtained from the A. S. Aloe Co., 1819 Olive St., St. Louis, Mo.

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THE CHEMICAL DIAGNOSIS OF PREGNANCY BY DETECTION OF ESTRIN IN URINE*

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DURING the course of an investigation of the phenol excretion in pregnancy, we have devised a short method of chemically detecting estrin in the urine of pregnant women. The method consists in the extraction of estrin from the urine with ether and its detection by coupling with diazotized para-nitroaniline (the color reagent) to form a deep colored azo dye, a reaction first noted by Harington and Schüpbach.[1] The term *estrin* is used in this paper to include *theelin* (ketohydroxyoestrin) and *theelol* (trihydroxyoestrin) and is synonymous with *female sex hormone, folliculin, menformon,* and *progynon.*

A chemical test for estrin in the urine of pregnant mares[2] has appeared while we were working, based on the extraction of estrin and its reaction with concentrated sulphuric acid to give a fluorescent greenish red color. This reaction is a well-known one, and was first used by Kober[3] in devising a method for the chemical assay of pure theelin. The Kober assay has recently been studied and modified by Cohen and Marrian,[5] and a method for the quantitative chemical estimation of theelin and theelol in urine of pregnant women has been devised by these investigators. We have studied the sulphuric acid fluorescence reaction with human urine extracts prepared as described below and found that it gives a color which is far less distinct and less sensitive, and by far less easy to estimate colorimetrically, than that given by the diazo reaction.

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An extract from a paper presented, by invitation, before the Baltimore City Medical Society, Section of Obstetrics and Gynecology, March 8, 1935.

The substances ether-extractable from acidified urine which may react with the color reagent are, essentially, all phenols of different types: estrin, free volatile phenols (normally almost none), nonvolatile phenols, phenols conjugated with sulphuric and glycuronic acids, and aromatic hydroxy acids. These phenols are shown in analytic classification in Fig. 1.

REAGENTS EMPLOYED

Ether.—Merck's anesthetic ether (U.S.P. X) is used throughout.

Sodium Carbonate Solution.—Twenty per cent (wt. by vol.). Baker's technical anhydrous in distilled water. Sp. Gr. 1.1828 at 23° C.

Saturated Sodium Carbonate Solution.—Baker's technical anhydrous in distilled water. Sp. Gr. 1.22 at 23° C.

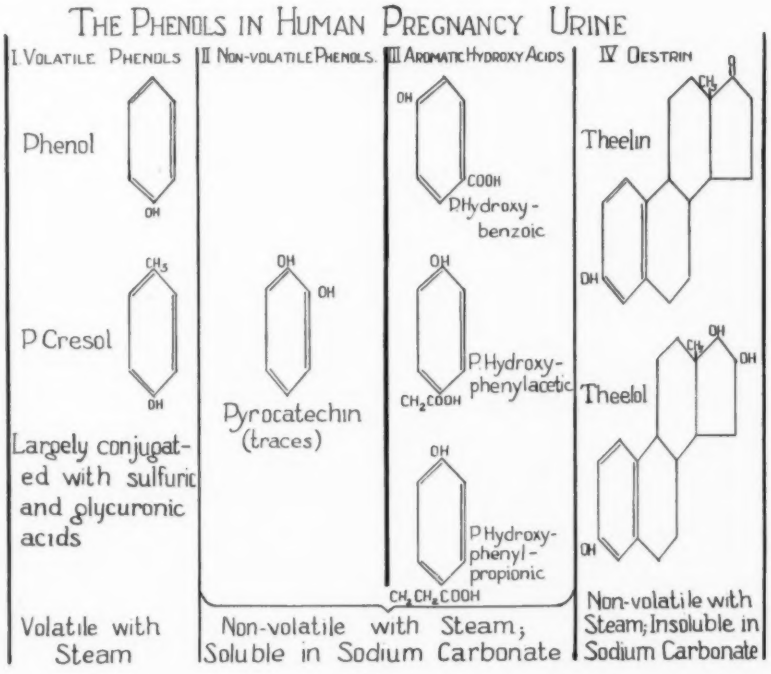

Fig. 1.

Stock Sodium Nitrite Solution.—Five per cent. Twenty-five grams of Baker's analyzed 86.3 per cent $NaNO_2$ dissolved in water and diluted to 500 c.c.

Stock p. Nitroaniline Solution.—Dissolve 1.5 gm. p. nitroaniline (Eastman, No. 179) in 500 c.c. of water containing 40 c.c. of conc. HCl.

Ferric Chloride Solution.—Thirty-three per cent. Five hundred and fifty grams of $FeCl_3.6H_2O$ (Baker's Analyzed) dissolved in water and diluted to 1,000 c.c.

PREPARATION OF P. NITRODIAZONIUM CHLORIDE SOLUTION

(The Color Reagent)

Twenty-five cubic centimeters of the stock p. nitroaniline solution are added to 1.5 c.c. of the stock solution of sodium nitrite. This solution is prepared fresh daily. At room temperature or in ice water, this solution slowly becomes cloudy, but in the refrigerator at about 5° C. it remains clear indefinitely.

METHOD AND PRINCIPLES

The twenty-four hour urine specimen is brought to pH 4 with concentrated hydrochloric acid and evaporated down to about 200 c.c. This treatment "deconjugates" the conjugated volatile phenols and volatilizes almost completely the volatile phenols thus formed. This volatilization has been verified by control experiments with pure solutions of phenol and p-cresol.

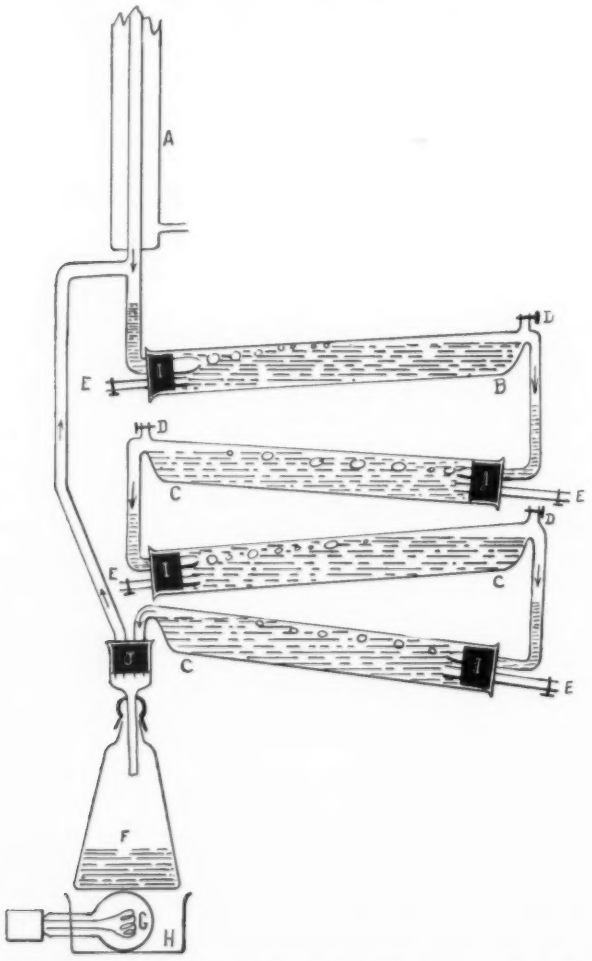

Fig. 2.—*A*, Condenser; *B*, Urine extraction tube, 300 c.c. capacity; *C*, Sodium carbonate washers, 100 c.c. capacity; *D*, Air vents; *E*, Inlet-outlet pinch-clamps; *F*, Ether; *G*, 60-watt lamp; *H*, Asbestos-lined can; *I*, Rubber stoppers; *J*, Cork stopper.

The acid hydrolysis also releases the hormone from an ether-insoluble ester combination (probably with sulphuric acid) as Marrian[4] and Cohen and Marrian[5] have shown.

The urine is then brought to pH 1 with concentrated hydrochloric acid, since it has been shown[6, 7] that the extraction is more efficient at this concentration, and the hormone, together with the remaining phenols, is then extracted with 150 c.c. ethyl ether. For this purpose we have devised an

apparatus (Fig. 2) which not only continuously extracts the urine but also automatically washes the ether extract with sodium carbonate solution, thus removing most of the aromatic hydroxy-acids and pyrocatechin. We generally allow the extraction to proceed from 5 P.M. one day to 9 A.M. the next.

The ether extract is washed with 10 c.c. portions of 20 per cent sodium carbonate until no further change is seen when 1 c.c. of the color reagent is added to the wash, and then with saturated sodium carbonate until the wash gives a negative test with 1 c.c. of the color reagent. This washing quantitatively removes aromatic hydroxy-acids, as Tisdall[8] has shown. We have found that it also removes pyrocatechin. However, sodium carbonate does not remove estrin from ether for it is used in this way in many isolation procedures.[4] A 10 c.c. wash with 2 per cent hydrochloric acid and a 10 c.c. wash with distilled water conclude the washing of the ether.

Volatile phenols still remaining in the ether extract are now removed by distilling off the ether, adding water to the residue, and distilling until the distillate (made alkaline with sodium carbonate) no longer gives a positive reaction with the color reagent. This procedure quantitatively volatilizes phenol and cresols as Hanke and Koessler[9] have shown. The last traces of water are removed by heating in a glycerin bath at 160° C.

The hormone residue is now dissolved in 7.5 c.c. of 95 per cent ethyl alcohol with warming. This is cooled, 1 c.c. of the color reagent is added, and 15 c.c. of 20 per cent sodium carbonate are added. The flask is well swirled and the contents are transferred to an 8 × 1 inch test tube. The alcohol-carbonate phases spontaneously separate and the presence of estrin is indicated by a dark orange to deep wine-colored alcoholic layer over a colorless carbonate layer. When the urine contains very little estrin, as in the urine of males and nonpregnant females, the alcoholic layer is yellow to pale brown with no red tint.

For the purpose of permanent record, the colored alcoholic layer is carefully pipetted off, filtered into a dry colorimeter cup, and placed at 10 mm. This is matched against a 33 per cent ferric chloride solution, the reading of which is recorded as the "ferric chloride number" (F.N.). In late pregnancies in which the estrin excretion is accordingly higher it is sometimes necessary to place the colored alcoholic test solution at 5 or 2.5 mm.; such readings are, respectively, doubled or quadrupled. For purpose of pregnancy diagnosis, we may say that an F.N. below 15 is considered negative and above 25, positive. Numbers between 15 and 25 are doubtful and have been obtained in very early pregnancy, in cases of dead fetus, and in 1 case of menopause.

RESULTS

Our chief interest in this method has been for the chemical diagnosis of pregnancy, for which purpose we have used it in various forms, for the past year and a half. The results of 89 urinalyses made on 56 pregnant and nonpregnant individuals are summarized in Table I. The detailed clinical results will be discussed more fully elsewhere.

An especially interesting fact is that we have been able to confirm Smith and Smith's[12] finding of a considerably diminished excretion of estrin in the toxemias. Our series includes 5 cases of prepartum and intrapartum eclampsia and the estrin excretion in these cases has been even lower than that of twelve-week normal pregnancies. In cooperation with the Department of Obstetrics of the University Hospital, we are now studying the value of this test as a method of diagnosing impending eclampsia, and the effects of theelin treatment in such cases.

TABLE I

	56 INDIVIDUALS	89 URINALYSES
Known Pregnant	38	58
Positives	37	53[6]
Doubtfuls	1[1]	5[2]
Negatives	0	0
Known Nonpregnant	22[3]	31
Negatives	16	24[7]
Doubtfuls	5[4]	6[4]
Positives	1[5]	1[5]

[1]Two or three weeks pregnant, but not confirmed clinically until two months later. A later specimen not available.

[2](1) – Same as [1] above.
(2) – Doubtful at four days past the expected menses, positive ten days later.
(3) – Doubtful at ten weeks, bleeding one day monthly, positive at twelve weeks, therapeutically aborted at fourteen weeks.
(4, 5) – Aged forty-two years, doubtful tests at seven and eight and one-half weeks, Mazer-Hoffman test negative; positive at eleven weeks.

[3]Includes four postpartum urines of individuals also studied before delivery.

[4]Six urines of five patients:
(1, 2, 3) – Missed abortions, two before and one after delivery.
(4) – This patient had in the left lower quadrant of the abdomen a twenty-six-week-old fetal skeleton which had been there for three years.
(5) – Menopause, nervous symptoms intense.
(6) – Ten days postpartum.

[5]Six week menstrual cycle, 4,760 c.c. (48 hr. spec. ?), two days before menses.

[6]Distribution of 53 pregnancy urines.

9	– 3 to 4 wk. pregnant
8	– 5 to 8 wk. pregnant
21	– 3 to 6 mo. pregnant
14	– Before delivery
1	– 16 wk. pregnant but menstruating regularly
53	

[7]Distribution of 24 negative urines.

2	– Normal, nonpregnant females
5	– Normal males
4	– Menopause
1	– Missed abortion, before delivery
1	– Missed abortion, after delivery
5	– Normal postpartums
1	– Large ovarian cyst
1	– Uterine fibroid
1	– Cervical polyp
1	– Ectopic pregnancy, two days preoperative
1	– Menses delayed eight days
1	– Endocrinopathy
24	

DISCUSSION

The sensitivity of the diazo coupling reaction may be recognized when we recall that Hanke and Koessler[9] showed that as little as 0.001 mg. of phenol can be estimated quantitatively with it (they used diazotized sulphanilic acid). Furthermore, examination of Hanke and Koessler's data will show that the reaction is stoichiometric; that is, equimolar quantities of different phenols give the same amount of color production. Hence, theelin and theelol, with a molecular weight 3 times that of phenol, theoretically should give an estimable color production in quantities as small as 0.003 mg. (We have found, in work in progress on a quantitative chemical method of estimating estrin, that 5 rat units of pure theelin gave a quantitatively measurable color production.) Now, when it is recalled that Cohen and Marrian[5] have shown that urine of women late in pregnancy contains 1.0 mg. theelol and 0.1 mg. theelin per liter, and that even women in the first six weeks of pregnancy excrete 300 to 600 mouse units of estrin,[10] the depth and sensitivity of this reaction is not surprising.

In addition to the acidic phenols, neutral substances are also ether-soluble from acid urine. "The two principal neutral substances which are so far known to occur in human pregnancy urine (are) cholesterol and pregnandiol."[5] Cholesterol was tested and found not to react with the color reagent; pregnandiol was not available for testing but would hardly be expected to react.

Histidine, which has been found in pregnancy urine in increased quantities,[11] has also been subjected to our procedure. As we expected, it was found to be ether-insoluble from the hydrochloric acid in which it forms the hydrochloride. This is true of the aromatic and heterocyclic amino acids in general.

Volatile phenols were originally removed from the ether by washing with 2.5 per cent sodium hydroxide. Although extracts so washed were still decidedly estrogenic, it is quite likely that this wash removed most of the theelol, for Cohen and Marrian[5] have shown that N/10 sodium hydroxide removed theelol from ether almost quantitatively while N/1 sodium hydroxide (about 4 per cent) is required to remove theelin. The first thirty-two urinalyses were done by the sodium hydroxide-wash method and the "ferric chloride numbers" given by the pregnancy urines in that series are considerably lower than those given with the steam distillation method used thereafter, evidence of the fact, we believe, that theelol was removed by 2.5 per cent sodium hydroxide.

The ether extract is generally colored light yellow by urine pigments, and this color largely persists even after the washing. Treatment of the extract with Norite A (Pfanstiehl) completely removes the pigments, but, judging from the color tests obtained with divided pregnancy extracts in which one portion was so treated, we believe hormone is also lost. We, therefore, do not advocate its use at present.

SUMMARY

A method is described for the chemical detection of theelin and theelol in urine of pregnant women. Results obtained with this method in 89 urinalyses of 56 patients suggest that it may be used for chemical diagnosis of pregnancy.

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Note added in proof, November 1, 1935: Since this paper was accepted for publication, several small modifications of the procedure have been made. We now use a much simpler extraction-washing apparatus—a description of this will appear in *Science*. The washing of the extract is done with a stirrer in a cylindrical separatory funnel. The final drying is carried out in a water-bath under vacuum rather than in the glycerin bath at 160° C. These changes described in detail together with results on 200 urinalyses to date will be the subject of a subsequent paper.

The authors will be glad to send a description of these changes in procedure to anyone on request.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

BANTI'S DISEASE, Relation of Chronic Congestive Splenomegaly to, Larrabee, R. C. Am. J. M. Sc. **188**: 745, 1934.

On the basis of a study of 47 cases the view is expressed that in the majority of patients presenting the clinical picture of Banti's disease (splenomegaly with fibrosis, microcytic anemia with leucopenia and a late stage with hemorrhages and ascites) the condition is dependent upon various intraabdominal lesions obstructing the venous outflow of the spleen. By far the commonest of these is liver cirrhosis of various types. As Banti has limited the definition of the disease which bears his name in such a manner as to exclude these cases it is thought best to segregate them under a distinctive name. However, splenectomy is indicated just as in Banti's disease, regardless of the nature of the underlying lesion.

JAUNDICE, Study of One Hundred Cases of, With Particular Reference to Galactose Tolerance, Schiff, L., and Senior, F. A. J. A. M. A. **103**: 1924, 1934.

The galactose tolerance test was positive (output of galactose exceeded 3 gm.) in forty-nine of fifty cases of catarrhal jaundice and fourteen of fifteen cases of acute toxic hepatitis.

A negative test (output of less than 3 gm.) was obtained in all of twenty cases of obstructive jaundice. Negative tests were mostly obtained in cases of cirrhosis and neoplasm of the liver.

The test appears of great value in differentiating acute (toxic or infectious) jaundice from obstructive (extrahepatic) jaundice.

The galactose tolerance bears no direct relationship to the degree or duration of jaundice or to the amount of retention of bromsulphalein. In the later stages of acute intrahepatic damage it may remain positive when other tests of liver function have become negative.

The test should be repeated when a discrepancy arises between clinical and laboratory observations.

SPUTUM, Study of, in Pulmonary Asbestosis, Page, R. C. Am. J. M. Sc. **189**: 44, 1935.

From a study of 38 cases the author concludes that:

1. The presence of asbestos bodies in the sputum is indicative merely of exposure to asbestos dust. If they are of large size it means that a long interval has elapsed since the onset of exposure.

2. The number of bodies in any given specimen is insignificant, but the presence of old and weathered bodies on repeated examinations strongly suggests that a definite pathologic process is in existence.

3. Clumps in the sputum are definite evidence of lung disintegration, but their absence does not mean that disintegration is not in process.

4. Elastic fibers are probably indicative of rapid lung destruction.

5. Elastic fibers may be present in the sputum in pure pulmonary asbestosis both with and without clumps, or in asbestosis with associated tuberculosis.

6. The routine examination of the sputum in cases of suspected pulmonary asbestosis is essential, as it plays a significant rôle in the clinical diagnosis.

SEDIMENTATION TEST, Standardized Technic for, Wintrobe, M. M., and Landsberg, J. W. Am. J. M. Sc. **189:** 102, 1935.

The following method is recommended as satisfactory and accurate:

1. Five cubic centimeters of venous blood is collected by means of a dry syringe and needle and mixed in a small bottle containing 4 mg. solid potassium oxalate and 6 mg. solid ammonium oxalate. This concentration of oxalate does not alter the sedimentation rate as compared with that of blood collected in heparin. Less than 1 c.c. of blood is needed for the sedimentation test. The remainder can be used for other blood examinations.

2. The blood so collected should be used for the determination of sedimentation rate within four hours of its time of collection. Further delay may be associated with increased suspension stability of the blood.

3. The hematocrit is filled to the 10 cm. mark with blood. The upper level of sedimenting corpuscles may be read at frequent intervals or, more simply, a single reading may be made at the end of 1 hour.

4. Since sedimentation rate increases with increasing temperature, the sedimentation test should be carried out at a temperature not less than 22° nor greater than 27° C. Within this range variations resulting from differences in temperature are small. If the blood used has previously been kept in a refrigerator it should first be permitted to attain the above temperature before being used.

5. The hematocrit should be kept in an exact vertical position during the sedimentation of the blood corpuscles, for when the instrument stands at an angle of even 3° from the vertical, significant acceleration of sedimentation takes place.

6. After sedimentation rate has been determined, the hematocrit containing the blood should be centrifugalized and volume of packed red cells determined. The sedimentation rate may then be corrected for alterations due to anemia. A correction chart is presented.

PNEUMOCOCCUS, Sodium Desoxycholate for the Identification of, Leifson, E. J. A. M. A. **104:** 213, 1935.

Method: 2 drops of a 10 per cent aqueous solution of sodium desoxycholate are added to 1 c.c. of pneumococcus culture. Lysis occurs in two to five minutes. The pH of the culture must not be below 6.5 or the reagent precipitates, and the temperature at which the test is carried out should be below 50° C.

(The reagent can only be obtained in this country from Riechel-deHaen, New York). The reaction is not seen with any strain of streptococci thus far tested.

PNEUMOCOCCUS AND STREPTOCOCCUS VACCINES, Cutaneous Reactions of Children to, de Bruin, M., and Vedder, A. Am. J. Dis. Child. **48:** 791, 1934.

Infants younger than three months react weakly or not at all to intracutaneous injections of vaccines of dead pneumococci and streptococci.

Older infants and young children react in a gradually increasing percentage of cases.

No type-specific or species-specific differences between the vaccines were observed. Vaccines of pneumococci and green-producing and hemolytic streptococci act the same.

The reaction is specific for the group of pneumococci and streptococci.

Children who have suffered from bronchitis, lobular pneumonia, and other conditions in which pneumococci and streptococci are of etiologic importance often give a positive reaction; other children, a negative reaction. Possibly as a result of such a condition a negative reaction changes into a positive one.

Older children and adults (students) in the majority of cases react positively.

Children who have suffered from lobar pneumonia, acute rheumatic fever and chorea almost never react to the vaccines.

In persons having suffered from erysipelas the percentage incidence of negative reactions is the same as the chance of recurrence.

In erysipelas a positive reaction points to immunity; a negative one indicates that a chance of recurrence exists. In lobar pneumonia and rheumatic fever also some relation perhaps exists between a negative reaction and the incidence of recrudescence.

The skin reaction has a significance analogous to the tests of Pirquet and Mantoux.

GONOCOCCUS: The Criteria of Cure of Gonorrhea in the Male, King, A. J. J. A. M. A. 104: 178, 1935.

Attention is called to the value of the oxidase test in the presumptive recognition of gonococcus colonies.

Flood the surface of the medium with a *freshly prepared* 1 per cent solution of dimethyl-paraphenylenediamine hydrochloride in distilled water.

Colonies showing the characteristic color reaction (a pink coloration deepening through purple to jet black in about thirty minutes) are selected for gram-staining. The test is not specific, other members in the neisserian group reacting, but only the *M. catarrhalis* closely simulates the reaction given by the gonococcus.

False positive reactions are usually given by a thin filiform type of *B. coli* and also by *B. subtilis* but easily detected on microscopic examinations.

EMPYEMA, Putrid, With Special Reference to Anaerobic Streptococci, Fisher, A. M., and Abernethy, T. J. Arch. Int. Med. 54: 552, 1934.

Attention is called to the fact that infections due to anaerobic streptococci may occur frequently and fail to be recognized unless cultivation is properly performed.

A case is reported of putrid empyema with pulmonary abscesses septicemia and metastatic putrid abscesses due to a gas-producing, facultative anaerobic streptococcus.

Other cases of putrid empyema are reported in which an anaerobic streptococcus was found in conjunction with other organisms.

Anaerobic streptococci were found to be the predominant organisms in 2 cases of pulmonary abscess in which cultures were made directly from the pus at operation. Other authors have stressed the frequent occurrence of these organisms in such lesions.

Although the finding of anaerobic streptococci in cases of pleural and pulmonary disease has been emphasized, the literature indicates their frequent occurrence in other infections, notably in cases of puerperal sepsis or septic abortion, otitis media, pulmonary abscesses, and hepatic abscesses.

Mention is also made of a case of pyelo-phlebitis with multiple hepatic abscesses due to an anerobic streptococcus. An attempt is made to identify the streptococci reported with various types described in the literature. They do not completely fulfill the characteristics of any one group.

Cultures of one strain were capable of producing empyema in rabbits when injected intrapleurally. Other strains gave varying results under similar conditions.

Evidence is presented to indicate that these streptococci may often be pathogenic for man and for animals.

BREAST LESIONS, Clinico-Pathological Relationship in Common, Lamb, F. H. Am. J. Clin. Path. 4: 327, 1934.

A knowledge of the clinicopathological relationships in breast disease is the basis for its intelligent management. The simple classification of benign and malignant tumors, cystic and inflammatory disease covers the vast majority of lesions with which one may be confronted. Some of the natural limitations in the differential diagnosis of the most common breast lesion, namely the solitary mobile nodule, have been emphasized in a statistical way. It has been pointed out that multiple lesions in one or both breasts are likely to be benign solid tumors of a type of cystic disease, and the rationale of simple

excision in the former case and mastectomy in the latter has been explained. Personal experience in the immediate examination of breast tumors has demonstrated repeatedly the advantage of this form of cooperation between the surgeon and pathologist. An exploratory operation on the breast should not be undertaken unless one is fully prepared to perform a radical operation if malignant disease is found.

BRONCHIECTASIS, Morphologic Varieties of, in the Adult, Bendove, R. A., and Gershwin, B. S. Arch. Int. Med. **54**: 131, 1934.

Recent studies of the respiratory dynamics of the bronchi and their defensive or expulsive mechanism are briefly reviewed and the physiopathologic changes which may precede or accompany bronchial dilatation are discussed with especial reference to their diagnostic significance in early bronchiectasis.

Six distinct morphologic types of bronchial dilatation, five of which are bronchiectatic and one of which is bronchiolectasis, are described as visualized roentgenologically after the intrabronchial injection of iodized oil. They are: the tubular, the cylindric, varicose and globular types, bronchiolectasis and bronchiectatic abscess.

The various theories concerning the mode of production of the polymorphic varieties of bronchiectasis are advanced, and the probable pathogenesis of each type is discussed. The tubular form may result from prolonged irritation of the bronchial mucosa; sclerotic and degenerative changes of the myo-elastic layers are considered to be the primary lesion in the cylindric form; the varicose type is, as a rule, brought about by massive pulmonary cirrhosis or atelectasis, whereas a diffuse bronchopulmonary distribution of fibrosis may entail bronchiolectasis; the globular type is only a segmental pouching occasioned by local weakening in the bronchial wall and the primary bronchiectatic abscess is initiated by suppurative lesions in the bronchi.

Each of these morphologic types reveals itself clinically by a different mode of onset, a variable train of symptoms and certain physical signs. The clinical history is of greater diagnostic value than are the physical signs. Further studies and closer correlation between symptoms, signs and morphologic aspects as outlined by iodized oil in the living will no doubt enable us to draw a sharper line of clinical demarcation between the various forms.

No diagnosis of bronchiectasis is complete unless the location, the distribution and, above all, the type have been determined, for on this information often depends the therapeutic procedure to be followed.

BLOOD: Relationship of the Intrinsic Factor to a Hematopoietic Material in Concentrated Human Gastric Juice, Helmer, O. M., Fouts, P. J., and Zerfas, L. G. Am. J. M. Sc. **188**: 184, 1934.

This is a continuation of previously reported studies upon the increased potency of liver extract No. 343 after incubation with 100 c.c. of normal gastric juice. (Minot, G. R., and Murphy, W. P., J. A. M. A. **87**: 470, 1934.

The purpose of the present investigation was to study the intrinsic factor responsible and to attempt to determine some of its characteristics. The following conclusions are advanced:

The active principle necessary for the maturation of red cells is present in the liver at least 2 months before birth.

The active principle may be absent from the liver of an inadequately treated case of pernicious anemia.

The active principle is present in the liver of an adequately treated case of pernicious anemia.

A cirrhotic liver may not contain the active principle.

It is highly suggestive that a liver may be sufficiently damaged so that the active principle, though present, cannot be presented to the tissues for utilization.

A pernicious anemia-like blood picture may be present in a patient, if the liver is so damaged that it cannot store the active principle, or cannot present it to the body tissues in the proper form for utilization.

The demonstration that all livers do not contain the "active principle" indicates that it is a storage product rather than an intrinsic part of the liver substance.

RHEUMATOID ARTHRITIS, Treatment of Chronic, With Streptococcus Vaccine, Wainwright, C. W. J. A. M. A. **103:** 1357, 1934.

Ninety-four blood cultures in 91 cases of rheumatoid arthritis yielded *Streptococcus viridans* in 1 case, diphtheroids in 4 cases, staphylococci in 4 cases and gram-positive bacilli in 3 cases.

Fourteen joint cultures in 14 cases of rheumatoid arthritis were negative.

The serums of 46, or 90 per cent, of 51 cases of rheumatoid arthritis were found to possess agglutinins for hemolytic streptococci.

All of 55 cases of rheumatoid arthritis gave positive skin reactions to one or more strains of streptococci.

Twenty-one of 28 cases of rheumatoid arthritis have shown improvement following intravenous injections of streptococcus vaccine prepared from the strain to which the skin was most sensitive.

CANCER, Roffo's Test in. Statistical Results of 1,100 Cases, Gandolfo, A. Am. J. Cancer **22:** 363, 1934.

Though Roffo's test is not specific, it is of value as an auxiliary method in the diagnosis of cancer.

On account of the simplicity of its technic it can and should be currently used in clinics.

Though a negative Roffo's test does not exclude the presence of a tumor, a positive result should induce us to continue investigations, in order to discover the tumor, as the proportion of erroneous positive results in this test is small (6.37 per cent in 6,718 tests undertaken).

Roffo's test has given a high percentage of positive results in cancer of the uterus (68.33 per cent), ovary (78.94 per cent), bladder (72.41 per cent), stomach (76.26 per cent), intestine (76 per cent), liver (84.20 per cent), pancreas (84.61 per cent), lungs (75 per cent), and mediastinum (86.66 per cent), and in osteosarcomas (71.42 per cent), all of which generally offer greater difficulties in clinical diagnosis.

ALBUMINURIA: Functional, Analysis of 58 Cases With Results of Thyroid and Calcium Medication, Burden, N. J. Am. J. M. Sc. **188:** 242, 1934.

Nothing was found in the past medical histories or in the physical examinations of 56 otherwise healthy male students with persistent functional albuminuria to account for the urinary abnormality. The kidney function as determined by the phenolsulphonephthalein and Mosenthal tests was normal.

Cardiorenal disease in the parents of students with functional albuminuria was more than twice as frequent as in the parents of students showing no such abnormality.

A tendency to low basal metabolism readings was found in 80.9 per cent but in the 10 cases tested with moderate doses of thyroid extract there was no improvement in the albuminuria.

The blood serum calciums were normal in 10 students with persistent functional albuminuria, although calcium medication had a favorable influence on the albuminuria in 11 of 16 cases tried.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Disorders of the Blood*

DESPITE the importance of the blood as a specialized tissue, primary disease of the blood per se is relatively uncommon. On the other hand, the varied functions of the blood as the great common carrier of the body render inevitable its association with disease in general so that changes in the peripheral blood constitute important evidence of disease or disorder of other systems of the body.

The title of this book, *Disorders of the Blood* has been chosen with this thought in mind and indicates the comprehensive nature of its contents.

The volume begins with a discussion of the origin, developments, and functions of the blood cells and also of the abnormal cells found in the circulation. This is followed by the principles and practice of hematologic diagnosis. This is followed by discussions first of diseases arising primarily in the blood itself and the hematopoietic system, and then by discussions of the various changes associated with other diseases as a reflection or indication of which disturbances occur in the blood.

The authors have attempted to reduce the hematologic literature of the past decade to a reasonable compass but, when necessary, have not hesitated to comment on or expand it from the aspect of their own experience which, obviously, has been extensive.

At the end of each chapter the contents are summarized briefly and to the point.

The style is clear and without verbosity or ambiguity. The illustrations are well chosen and reproduced.

An excellent index and a separate index of authors complete a well-planned and well-executed volume which may be recommended heartily as an authoritative and comprehensive reference text upon disorders of the blood.

Clinical Diagnosis by Laboratory Methods†

TODD and Sanford's *Clinical Diagnosis by Laboratory Methods*, used for years, certainly needs no introduction.

The eighth edition is outstanding with its several advances. First, rearrangement of the chapters and bringing the subject matter together more compactly and intelligently is a vast improvement. Second, the book has necessarily been enlarged to include entirely new methods and improved illustrations. Of particular interest is the new section on hemoglobin, including the Wintrobe, Haden-Hausser and Osgood Haskins hemometers. The new chapter on clinical chemistry, including determination of sulphemoglobin, the Exton and Rose method for sugar tolerance, a simple test for carotinemia, cholesterols, lecithin and total lipid in blood plasma will be found very useful.

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*Disorders of the Blood, Diagnosis, Pathology, Treatment and Technic. By Lionel E. H. Whitby, Assistant Pathologist, The Bland-Sutton Institute of Pathology, etc. and C. J. C. Britton, Assistant Pathologist, The Christchurch Hospital, New Zealand. Cloth, pp. 543, 12 colored plates, 53 figures. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

†Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology. By James Campbell Todd, Ph.B., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine, and Arthur Hawley Sanford, A.M., M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation); Head of Section on Clinical Laboratories, Mayo Clinic. Eighth edition thoroughly revised with 370 illustrations, 29 in colors, pages 792. W. B. Saunders Company, Philadelphia, 1935.

In addition there are such valuable addenda as Van Allen's thrombocytocrit, the Nygaard plasma platelet count, the Wintrobe and Landsberg sedimentation method, the rose bengal test, a quick method for preparation of colloidal gold as well as new methods in serology.

There are twenty-five new illustrations, well placed.

Clinical Atlas of Blood Diseases*

THOSE familiar with this very useful atlas will welcome its third edition. Those who are not will find it of great and valuable assistance in the study of diseases associated with disturbances of the blood.

As advances in the field of hematology have been greater in the clinical than in the morphologic field, the plates of this edition are the same. The text, however, has been revised and largely rewritten. It may be questioned whether the exceedingly brief section on hematologic methods which has been added increases the value of the book appreciably because of its brevity. With this minor criticism, the volume remains as before, a practical, useful and valuable contribution to the study of the blood.

Doctors and Juries†

IN THIS small book, the author has compressed more sound advice based on common sense and practical experience than appears, on the surface, in many larger volumes covering similar ground.

As expressed in the preface: "This book is for doctors who desire to know something of the legal aspects of their profession, and for lawyers and others who wish information concerning the relationships of the law with the practice of medicine."

It is to be hoped that among these it will have a wide circulation for it well repays reading and rereading by all who appear in court—not only the doctor who makes practically a business of such appearances, but especially by those who may, and often do, find themselves in court unexpectedly or even unwillingly.

While the author, as a lawyer, may not, perhaps, subscribe without reservation to the famous dictum laid down by Mr. Bumble, to wit: "The law is a ass!" he has not hesitated to comment upon the absurdities consequent upon legal technicalities.

To this reviewer, at least, it is refreshing to read a book on this subject which does not appear to be based upon the premises that all courts are majestic in their operation, all judges learned, and all juries intelligent, not to mention the assumption that all doctors are necessarily good or useful witnesses.

If all physicians appearing or about to appear in court were thoroughly familiar with the chapters on Testifying, Expert Witnesses, Hypothetical Questions, and Influencing Juries, the spectacle would be much improved in dignity and effectiveness. And every physician, if he does no more, should read the chapter on Observations which, appropriately enough, begins with "keeping out of trouble."

This is a valuable book, not only for the doctor but for the lawyer as well.

Textbook of General Bacteriology‡

THIS volume needs no introduction. That it has now reached an eleventh edition is ipso facto evidence of its usefulness and worth.

The present edition has been extensively revised, largely rewritten, and entirely reset and the book now reflects the many important changes and additions which have occurred in this field since the last edition of four years ago.

*Clinical Atlas of Blood Diseases. By A. Piney, M.D., Assistant Physician, St. Mary's Hospital for Women and Children, London; and Stanley Wyard, M.D., Physician, The Cancer Hospital, London. Cloth, pp. 110, ed. 3, 34 plates in color, 4 figures. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

†Doctors and Juries. By Hymphreys Springstrun. Cloth, pp. 155. P. Blakiston's Son & Co., Philadelphia, Pa.

‡Textbook of General Bacteriology. Edwin O. Jordan, M.D., Professor of Bacteriology, University of Chicago and Rush Medical College. Cloth, ed. 11, pp. 825, Figs. 202. W. B. Saunders Co., Philadelphia, Pa.

As heretofore, Jordan's *Bacteriology* can be recommended as comprehensive, well planned, equally well executed, and authoritative.

It will without doubt retain the place it has won as a practical and useful standard reference text.

Textbook of Bacteriology*

THIS is an eminently practical volume the purpose of which is to present bacteriology in a form best suited for assimilation by the student first meeting the subject and the physician desiring to review its more important practical and clinical applications.

To this end controversial subjects and theoretical discussions give place to the practical phases of the subject: diagnosis, prognosis, etiology, collection of laboratory specimens, interpretation of laboratory findings, treatment, specific therapy, prophylaxis, and sanitary control. Of particular value to the practicing physician is the fact that technical processes are limited to those feasible for him to carry out; when more than office laboratory equipment or ordinary skill is required, he is instructed only in the manner of collecting the sample, transporting it to the laboratory, and of interpreting the results.

The book is well indexed and may be warmly recommended to the practicing physician as a very practical, useful, and, withal, sufficiently comprehensive text.

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*Textbook of Bacteriology. By Thurman B. Rice, M.D., Professor of Bacteriology and Public Health at the Indiana University School of Medicine. Cloth, pp. 551, Figs. 121. W. B. Saunders Co., Philadelphia, Pa.